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Frequent isolation of *Arthroderma benhamiae* from dogs with dermatophytosis

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Abstract

Background—Molecular analysis methods have led to many changes in the taxonomy of dermatophyte species.

Hypothesis/Objectives—We hypothesized that fungi displaying morphology consistent with a traditional identification of ‘*Trichophyton mentagrophytes*’ represent multiple species, consistent with the new taxonomy.

Animals—Fungal specimens (n = 20) were collected directly from animals with dermatophytosis, were among those submitted for diagnostic analysis, or were part of historic teaching collections.

Methods—Primers that amplified a portion of the 28S ribosomal RNA gene, and primers specific for a fragment from the internal transcribed spacer (ITS) region, were used for PCR amplification of genomic DNA. DNA sequences from the amplified products were compared to databases to identify the isolates.

Results—80% (n = 16) of the fungal isolates were identified as *Arthroderma benhamiae*; eight were collected from dogs. One isolate was identified as *Arthroderma vanbreuseghemii*, two were *Trichophyton erinacei*, and one was *Nannizziopsis (Chrysosporium) guarroi*, which likely was present as a saprophyte.

Conclusions and clinical importance—Frequent isolation of *A. benhamiae* from dogs suggests a greater host range for this fungus than reflected in the current literature. Our data also suggest the potential for geographic restriction of strain types within the species. Efforts to identify fungal isolates using molecular techniques create a better understanding of diversity and epidemiology of the dermatophytes.

Introduction

Fungal species classified as dermatophytes are the cause of dermatophytosis, more commonly known as ringworm. Dermatophytes can be highly contagious with spread between humans, between animals, or zoonotic transfer between animals and humans. Isolation and culture of the dermatophyte associated with a clinical condition is a prerequisite to its identification. Identification is often pursued with the intent of understanding the source of the infection and to prevent its spread. Identification historically, and even currently, is commonly accomplished by evaluation of colony and cellular morphology of the dermatophyte isolate.

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Molecular methods have been introduced more recently and provide the opportunity for a more detailed taxonomy of dermatophyte species. This area of study is dynamic, with species names in seemingly continuous flux as new insights are gained into the diversity of dermatophyte species. Gräser et al.¹ reviewed these concepts and proposed a polyphasic approach to dermatophyte taxonomy. They highlighted the idea that isolates more traditionally identified as '*Trichophyton mentagrophytes*' represent a more diverse taxonomic group: a species complex. The goal of this work was to use molecular identification methods on a collection of fungi that were isolated from clinical cases of dermatophytosis in animal species. We hypothesized that the isolates that fit the traditional description of '*T. mentagrophytes*' represent a more diverse range of fungal taxa.

Materials and methods

Fungal isolates (n = 20; Table 1) with colony and microscopic morphology that could fit within the broad designation of '*Trichophyton mentagrophytes*' were obtained from the Veterinary Teaching Hospital, the Veterinary Diagnostic Laboratory, and historical teaching collections. Medical records were available for 13 of the isolates; these were collected between 2006 and 2011. Teaching collection isolates dated back as far as 1989.

Genomic DNA was extracted from cultures grown on Sabouraud agar plates. Mycelium was homogenized by vortexing in the presence of glass beads similar to the method of Ninet et al.². The homogenate was extracted with phenol/chloroform/isoamyl alcohol, precipitated with ethanol, and further purified using a QIAamp DNA mini kit according to the manufacturer's instructions (Qiagen).

Genomic DNA from each fungal isolate was amplified by polymerase chain reaction (PCR) using primers specific to the internal transcribed spacer (ITS) region of the nuclear-encoded ribosomal RNA genes (rDNA) (primers 18SF1 and 58SR1³), as well as primers specific for the 28S rRNA-encoding gene (primers LSU1 and LSU2^{2,4}).

The PCR products were purified and their DNA sequences determined by the staff at a campus core facility. Multiple approaches were used to identify the isolates, based on their DNA sequences. BLAST searches (blast.ncbi.nlm.nih.gov/Blast.cgi) were completed. Although this approach was helpful, misleading results may be obtained because many sequences have been submitted to public databases without rigorous identification of the fungal isolate. To minimize this difficulty, ITS sequences were matched against the ITS rDNA barcode database (<http://www.cbs.knaw.nl/dermatophytes/BioloMICS.aspx>). This database contains sequences from fungal isolates that have been rigorously identified using methods such as mating to tester strains to define species⁵. As our identification results became more refined, we compared our data to reference sequences from type strains or carefully validated isolates,^{1,4,5} to lend additional confidence to our conclusions. Genus and species names used here followed conventions described by Gräser et al.¹

Results

Table 1 shows the results for molecular characterization of the 20 fungal isolates. Sixteen of the strains (3208, 3209, 3216, 3220, 3329, 3350, 3355, 3356, 3360, 3361, 3368, 3369, 3370, 3371, 3376 and 3378) were identified as *Arthroderma benhamiae*. ITS amplicon sequences (335 bp) from all 16 isolates were identical and a 100% match to the corresponding region from the *A. benhamiae* type strain sequence (GenBank accession number AB088677). The 28S sequences (313 bp) from all 16 isolates were identical to each other, but showed one mismatch to the reference sequence from a carefully characterized tester strain (accession number GU646875)⁴: our isolates were polymorphic at position 293 in this sequence,

showing A or G (single nucleotide code R), compared to G in the reference sequence. Collectively, these results provided confidence that the 16 isolates were correctly identified as *A. benhamiae*.

One of the fungal isolates (3210; from a chinchilla) was identified as *Arthroderma vanbreuseghemii* (Table 1). The ITS sequence for this isolate (343 bp) matched 100% in the overlapping region with the GenBank sequence AF170452. This sequence was derived from a fungal strain that was also isolated from a chinchilla (IHEM 10162)⁵. Strain IHEM 10162 provided fertile mating products with *A. vanbreuseghemii* tester strains⁵. The 28S sequence for strain 3210 (313 bp) was a 100% match with the corresponding sequence from strain CBS428.63, a strain recognized as *A. vanbreuseghemii* (*Trichophyton interdigitale*).¹

Two of the isolates (3211, 3362) matched sequences from *Trichophyton erinacei* strains. The ITS sequences (335 bp) from these strains were identical to each other. Searching the ITS barcode database showed 100% identity between our sequence and *T. erinacei*, which accounted for the top 10 matches in the list. Comparison of our sequence to GenBank accession number Z97997, which was cited as representative of the species,¹ further supported our conclusions. Because of ambiguities in the large public databases, searches with the 28S sequences from these isolates were not productive.

BLAST searches with the ITS (301 bp) and 28S (314 bp) sequences from isolate 3353 (collected from a dog) matched 100% with sequences from *Chrysosporium guarroi* strain CCFVB CH10 (GenBank accession numbers FJ839684 and EU018451, respectively). Also apparent on the search results were numerous sequences from additional isolates of the same species, now renamed as *Nannizziopsis guarroi*.⁶ Re-examination of the colony morphology for strain 3353 indicated its similarity to published *N. guarroi* images.⁶

Discussion

The results showed the utility of DNA sequence analysis to identify and differentiate between isolates that fit into the traditional category of *T. mentagrophytes*. Considerable effort has been dedicated to understanding the taxonomy of this group of fungi in the recent past.^{1,4,5} Results here also display the complexities and dynamic nature of taxonomy for this group of fungi. In the current literature, manuscripts with the newest taxonomic designations are found side-by-side with those that use historical terminology. Conclusions regarding fungal diversity within a species and the epidemiology of the corresponding disease will gain clarity as the nomenclature becomes consistent.

The association between fungus and host species supported some previously reported relationships, as well as revealed some new trends. Consistent with our findings, *A. vanbreuseghemii* has been isolated from chinchillas,⁵ and *T. erinacei* has been isolated from hedgehogs.⁷ Isolation of *N. (Chrysosporium) guarroi* from a dog may be an incidental finding as *Chrysosporium* sp. may be saprophytic and unrelated to the dog's clinical presentation. *Nannizziopsis guarroi* is an emerging pathogen of reptiles;⁶ *Chrysosporium* sp. are known to produce a colour change on dermatophyte test medium, perhaps prompting preservation of the isolate from the primary culture.⁸

Results for the *A. benhamiae* isolates were the most novel. Current literature emphasizes the connection between *A. benhamiae* isolates and guinea pigs.⁹ This correlation emerged from a survey of 274 pets in Switzerland in 2006–2007. The study noted isolation of *A. vanbreuseghemii* from dogs and cats, but *A. benhamiae* only from guinea pigs. In our study, 80% of the fungal isolates were *A. benhamiae* (Table 1), with 80% of strains of known

origin collected from dogs. All of these dogs had a clinical presentation consistent with a diagnosis of dermatophytosis.

Arthroderma benhamiae taxonomy is gaining increased research attention. Symoens et al.⁴ completed additional taxonomic analysis of this fungal species, focusing on DNA sequence comparisons and mating interactions for strains that originated in Switzerland. Their work identified two groups among the Swiss *A. benhamiae* strains: group I (white colony type) and group II (yellow colony type). Our *A. benhamiae* sequences matched the ITS (AB088677) and 28S (GU646875) reference sequences for group II (yellow), however, our isolates have many, varied colony phenotypes, each of which more closely resembles the white type described for group I. Symoens et al.⁴ showed that this combination of sequence data and colony phenotype is typified by the most ‘classic’ mating tester strains for *A. benhamiae*, some of which date back to description of the *A. benhamiae* species in 1967.¹⁰ It is possible that *A. benhamiae* strains demonstrate geographic restriction and that our species are more typical of a North American isolate. Our frequent isolation of these strains suggests that they are common, and readily collected from dogs. Further analysis of these specimens will provide additional insight into the growing picture of strain diversity and epidemiology of *A. benhamiae*.

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Table 1

Summary of fungal isolates analyzed in this study

Morphological Identification	Molecular Identification	Source of Isolate				
		Cat	Dog	Chinchilla	Hedgehog	Unknown
<i>Trichophyton mentagrophytes</i> (n=20)						
	<i>Arthroderma benhamiae</i> (n=16)	1	9	2	1	7
	<i>Arthroderma vanbreuseghemii</i> (n=1)	1	8	1		6
	<i>Trichophyton erinacei</i> (n=2)			1	1	1
	<i>Nannizzopsis (Chrysosporium) guarroi</i> (n=1)		1			