

Case Report

Clinical manifestation of an amelanotic *Sporothrix schenckii* complex isolate in a cat in Germany

Sandra Scheufen,¹† Sellyn Strommer,²† Jascha Weisenborn,³
Ellen Prenger-Berninghoff,¹ Nina Thom,² Natali Bauer,² Kernt Köhler⁴
and Christa Ewers¹

Correspondence

Sandra Scheufen

Sandra.Scheufen@vetmed.uni-
giessen.de

¹Institute of Hygiene and Infectious Diseases of Animals, Justus-Liebig-University Giessen, Giessen, Germany

²Clinic for Small Animals, Department of Veterinary Clinical Sciences, Justus-Liebig-University Giessen, Giessen, Germany

³Institute for Ecology, Evolution and Diversity, Department of Mycology, J W Goethe-University, Frankfurt am Main, Germany

⁴Institute for Veterinary Pathology, Justus-Liebig-University Giessen, Giessen, Germany

Introduction: Dimorphic fungi of the *Sporothrix schenckii* complex are the aetiological agent of sporotrichosis in humans and animals. Cats in particular have gained importance in transmission of the disease to humans. Therefore, it is crucial to identify unusual aetiological agents and the varying clinical appearances of the disease.

Case presentation: We describe the case of a 10-year-old cat with a 2-month history of nasal discharge after a fight with another cat. Severe thrombocytopenia was diagnosed, whilst routine testing for feline leukemia virus (FeLV) was negative. A computed tomography scan revealed profound destruction of several parts of the nasal cavity. Histological and cytological examination of biopsies taken from these locations showed a chronic pyogranulomatous inflammation with several yeast-like structures. Mycological cultivation at 28 and 37 °C yielded fungal growth with smooth to wrinkled colonies consisting of hyphae and non-pigmented sympodial, round to oval-shaped conidia. Molecular typing, including sequence analysis of the ITS region, resulted in a consensus sequence which allowed classification of the fungus into the *Sporothrix schenckii* complex. Although the thrombocytopenia persisted, treatment with itraconazole dissolved any respiratory symptoms and improved the clinical status of the cat, indicating an antimycotic-responsive infection.

Conclusion: Amelanotic members of the *Sporothrix schenckii* complex should be considered a putative aetiological agent in the pathogenesis of feline sporotrichosis in Germany. Besides being classified in close proximity to species in the environmental clade this isolate has proven to cause serious infection implying a possible zoonotic potential.

Keywords: feline sporotrichosis; itraconazole; nasal discharge, thrombocytopenia; yeast-like organism.

Received 29 December 2014

Accepted 18 March 2015

Introduction

Sporothrix schenckii is a dimorphic fungus well known as the causative agent of sporotrichosis in humans and animals. Besides its presence as a geophilic fungus,

sporotrichosis has been reported in many different animal species with cats being suspected as the most prominent vector in transmission of the fungal infection to humans (Schubach *et al.*, 2005). One of the crucial pathogenic mechanisms of dimorphic fungi is their ability to change from a mycelial form at temperatures lower than 30 °C into a thermotolerant phase with yeast-like structures, which are capable of causing fixed cutaneous or occasionally systemic disease after infecting a host via wounds or scratches (Rodrigues *et al.*, 2013a; Welsh, 2003). Besides the dimorphism, melanization of conidia and the route of

†These authors contributed equally to this work.

Abbreviations: FeLV, feline leukemia virus; ITS, internal transcribed spacer; PAS, periodic acid–Schiff.

One supplementary figure is available with the online Supplementary Material.

infection are regarded as important aspects in terms of the clinical relevance of a strain, as a correlation with the degree of virulence has been observed (Arrillaga-Moncrieff *et al.*, 2009; Barros *et al.*, 2011; Brito *et al.*, 2007; Madrid *et al.*, 2010b; Nobre *et al.*, 2005).

Based on DNA analysis of the internal transcribed spacer (ITS) region and the calmodulin gene, the *S. schenckii* complex comprises a variety of strains usually divided into clades, which refer to their geographical distribution and their assumed medical importance (Rodrigues *et al.*, 2014; Zhou *et al.*, 2013). The following strains are usually regarded as clinically important members of the complex: *Sporothrix brasiliensis*, *Sporothrix globosa*, *Sporothrix luriei*, *Sporothrix mexicana* and *Sporothrix schenckii sensu stricto* (Marimon *et al.*, 2006, 2007; Zhou *et al.*, 2013). Except for *S. mexicana*, the four other species of the complex have been isolated primarily from clinical cases and therefore differ from environmental strains with a low potential to cause infection in mammals, such as *Sporothrix pallida* and *Sporothrix stylites* (Rodrigues *et al.*, 2013b; Zhou *et al.*, 2013). Furthermore, a correlation between species with higher virulence and geographical distribution has been supposed. Clinically relevant species of *S. schenckii*, for example, have a main focus in Brazil, whereas in Germany predominantly environmental species are mentioned (Zhou *et al.*, 2013). Regarding feline sporotrichosis, only a few cases have been reported in Germany (Weingart *et al.*, 2010).

The present case report of feline sporotrichosis caused by a member of the *Sporothrix schenckii* complex demonstrates the importance of considering amelanotic members of the complex in cutaneous lesions of cats, even in regions with a low prevalence of sporotrichosis.

Case report

Between March and August 2013, a 10-year-old rescued Persian-mixed breed cat was repeatedly presented to a university clinic for small animals due to a history of nasal discharge with involvement of initially the right and later the left eye after a fight with another cat in November 2012. Treatments with marbofloxacin and dexamethasone had shown no improvement. Instead, the nasal discharge worsened and became infiltrated with blood. According to the owner of the cat, there has been no stay abroad.

Clinical examination

Clinical examination of the cat revealed no severe abnormalities apart from repetitive sneezing and a nasal stridor as well as serous to haemorrhagic discharge from both nostrils. Along the bridge of the nose, a central dent was ascertainable. The right eye showed symptoms of mild conjunctivitis, viscous discharge and a dense mass measuring 0.5 × 0.5 cm on the nasal right orbita. Specific ophthalmological examination revealed a mild conjunctivitis of both eyes with epiphora particularly of the right eye and

an entropion of the lower eyelids. Further investigation of the area around the prominent mass by computed tomography scan showed a severe soft-tissue swelling with infiltration of the nasal cavity and destruction of the right os nasale, parts of the ossa maxillaria and the septum nasi.

For laboratory examination including cytology, histopathology and microbiology, blood and biopsy samples from the right side of the nasal bridge were taken and a FeLV test was performed (SNAP Combo Plus FeLV Ag/FIV Ab test; IDEXX Laboratories).

Cytology and histopathology

For cytological examination, several direct smears of the biopsies were stained with May–Grünwald–Giemsa stain (Merck) and microscopic examination was carried out. For histopathology, biopsies from the nasal cavity and biopsy material from the orbital mass were routinely fixed in 10 % formalin for about 24 h. Tissue samples were embedded in paraffin wax and sectioned at 4 µm using routine methods. Staining for light microscopy was performed with haematoxylin and eosin (H&E) as well as periodic acid–Schiff (PAS) in an automated stainer (HMS740; Microm).

Microbiology and molecular analysis

Bacteriological cultivation was performed on sheep blood agar, Gassner agar and two anaerobic media: Zeissler agar (Columbia agar base with D-(+)-glucose at 10 g l⁻¹) and Schaedler agar. The agar base was purchased from Merck and Becton Dickinson. For mycological cultivation, biopsy material was homogenized and streaked on modified Kimmig agar plates (15.0 g peptone l⁻¹, 1.0 g NaCl l⁻¹, 19.0 g D-(+)-glucose l⁻¹, 15.0 g agar agar l⁻¹, 5.0 ml glycerine/l, 50 mg Penicillin/l, 25 mg Streptomycin/l, pH 6.5) with and without cycloheximide (250 mg l⁻¹), modified Dixon agar (36 g malt extract l⁻¹, 6 g peptone l⁻¹, 20 g ox gall l⁻¹, 10 ml Tween 40/l, 4 ml olive oil, 20 g agar agar l⁻¹, 950 ml dH₂O) and brain–heart infusion agar (BHI) supplemented with 5% defibrinated sheep blood. Kimmig agar plates and Dixon agar plates were incubated for up to 26 days at 28 and 37 °C, whereas the BHI agar plates were incubated at 37 °C in CO₂-enriched (10%) atmosphere. Throughout the incubation period, the colonies that grew were examined phenotypically at least once a week, and adhesive tape or needle preparations with lactophenol cotton blue or phloxine were prepared for microscopic examination. To examine the ability of the fungus to produce melanin, it was cultured at 28 and 37 °C on plates containing a lower concentration of glucose (5 g l⁻¹).

For molecular analysis, DNA from the fungal colonies was isolated using hexadecyltrimethyl ammonium bromide (CTAB) (AppliChem) following a protocol slightly modified from Talbot (2001). In short, culture material of the fungus was homogenized and mixed with NaCl and CTAB lysis buffer. Several incubation and mixing steps were performed after addition of RNase, 2-mercaptoethanol and chloroform/

isoamyl alcohol. After centrifugation at 10 000 g for 10 min, the aqueous phase was collected and fungal DNA was precipitated by adding isopropanol. The pellet was washed repeatedly and finally resuspended in Tris buffer to be used for PCR. Regions of interest were amplified using primers ITS1f (Gardes & Bruns, 1993) and ITS4 (Innis, 1990) and KappaHifi polymerase (Peqlab) according to the protocol recommended by the manufacturer. Sequencing at GATC Biotech AG (Konstanz, Germany) and BiK-F (Biodiversity and Climate Research Centre, Frankfurt am Main, Germany) resulted in a consensus sequence (409 bp) of the ITS4 primer aligned with Geneious Pro 5.6.7 (Biomatters). For phylogenetic analysis the consensus sequence was aligned with ITS sequences published previously and listed in GenBank using MEGA6 (Tamura *et al.*, 2013). Identification numbers and the origin of the sequences are given in Table 1. For distance analysis, a minimum-evolution tree (Tamura–Nei model) with partial deletion of gaps and 1000 bootstrap replications was reconstructed (Fig. S1, available in the online Supplementary Material).

Investigations

Clinical findings

Haematological analysis revealed a severe thrombocytopenia (23000 l^{-1} ; reference range $180000\text{--}550000 \text{ l}^{-1}$) and a mild neutropenia ($2.1 \times 10^9 \text{ l}^{-1}$; reference range $2.5 \times 10^9\text{--}12.5 \times 10^9 \text{ l}^{-1}$). Clinical chemistry findings included mild hyperproteinaemia (84.7 g l^{-1} ; reference range $54.7\text{--}78 \text{ g l}^{-1}$) based on hyperglobulinaemia (53.4 g l^{-1} ; reference range $26\text{--}51 \text{ g l}^{-1}$) as well as mild hypokalaemia (3.45 mmol l^{-1} ; reference $3.6\text{--}4.8 \text{ mmol l}^{-1}$) and mild hypocalcaemia (1.12 mmol l^{-1} ; reference $1.19\text{--}1.41 \text{ mmol l}^{-1}$), whereas the FeLV/feline immunodeficiency virus status proved negative.

Cytology

Cytological examination yielded a picture of a severe pyogranulomatous inflammation with severe hyperplasia of plasma cells and several yeast-like structures (Fig. 1a).

Histopathology

In both localizations, a marked pyogranulomatous and chronic suppurative inflammation was observed (Fig. 1b) and there was a suspicion of unstained fungal stages within the lesion. Using special stains, a moderate number of fungal organisms were visualized, characterized by intensely PAS-positive oval yeasts of $3\text{--}6 \mu\text{m}$ and small hyphae (Fig. 1c, d).

Bacteriological and mycological findings

Bacteriologically, only poor growth of *Staphylococcus felis* was diagnosed, whereas mycological examination discovered pure growth of small white colonies with a smooth

and creamy surface already after 72 h incubation at $28 \text{ }^\circ\text{C}$ as well as at $37 \text{ }^\circ\text{C}$. During further incubation at $28 \text{ }^\circ\text{C}$, a white airy mycelial phase developed in the centre of the colonies (Fig. 2a, b) consisting of hyphae with conidiogenous cells and conidia lying in groups or in a sympodial order (Fig. 2c, d), whereas the smooth appearance remained visible in the peripheral region. Microscopically, hyphae as well as yeast-like organisms (Fig. 2e, f) could be found here despite the low incubation temperature. Colonies at $37 \text{ }^\circ\text{C}$ maintained their smooth character on BHI plates but got a wrinkled surface on Kimmig and Dixon agar (Fig. 2g, h). Microscopically, in these plates yeast-like structures dominated but were mixed with septate hyphae. Neither primarily nor after subcultivation or changing glucose concentration, was pigmentation observed.

BLAST searches (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) of the aligned sequence (409 nt) resulted in high similarity to several species, e.g. *S. pallida* (nucleotide identity 100%, GenBank accession no. KF574458), *Sporothrix* sp. (nucleotide identity 100%, GenBank accession no. GU129986) and *S. schenckii* (nucleotide identity 100%, GenBank accession no. AF484471), thus suggesting a classification of the fungus within the *S. schenckii* complex.

Phylogenetic analysis

Sequencing procedures resulted in the following consensus sequence: 5'-GCGCGTTTTGACGGCAGGGCGCCGGCGGGCGTCTGAGCGAGGGGAGAGAACTTGCGTCCAGTACTGCGCTCGGAGCCAACCAGCCGGGCCCGCCACTCGCTTTTCGGGGCCCTCCGCCAGGCGGAGGAGCCC CAACACCAGCGCGCAACGGGGCGCGTGAGGGGGG AAATGACGCTCGGACAGGCATGCCCGCCAGAATAC TGGCGGGCGCAATGTGCGTTCAAAGATTCGATGGT TCGCTGAATTCTGCAATTCACATTACGTATCGCATT TCGCTGCGTTCCTTCATCGATGCCAGAGCCAAGAGA TCCGTTGTTGAAAGTTTTGATTATTTGTTTTTAATCC TCAGACTGTTTCTAGTGGTTGAGATATAAAGGTTCCG TAGGGCCCGCCGCCCGGGCGGGCGGGGC-3'.

ITS sequences of 44 *Sporothrix* species from different countries were compared using a minimum-evolution tree with 1000 bootstrap replications (Fig. S1). The sequence obtained from this isolate was placed at a relatively short distance from species isolated from environmental sources, which is consistent with previously analysed isolates in Germany (Zhou *et al.*, 2013). Even though a strict separation between isolates from environmental versus clinical sources surrounding the sequence of this case was not evident, it seemed to be at a far distance from species of the complex building the clinical clade with higher pathogenic potential.

Treatment

The cat was treated initially with meloxicam (0.05 mg kg^{-1} *per os*, once a day) for 2 days. After that, itraconazole (5 mg kg^{-1} *per os*, once a day) was given daily for 5 months.

Table 1. *Sporothrix* spp. isolates included in the phylogenetic analysis

Species	Source	Country of origin	ITS GenBank no.	Reference
<i>Ophiostoma stenoceras</i>	Plant (<i>Eucalyptus smithii</i>)	South Africa	AY280491	Aghayeva <i>et al.</i> (2004)
<i>Ophiostoma stenoceras</i>	Plant (<i>Pinus ponderosa</i>)	USA	AF484476	de Beer <i>et al.</i> (2003)
<i>Sporothrix brasiliensis</i>	Human	Brazil	KC113211	Zhou <i>et al.</i> (2013)
<i>Sporothrix brasiliensis</i>	Human	Brazil	KC113212	Zhou <i>et al.</i> (2013)
<i>Sporothrix brasiliensis</i>	Human	Brazil	KC113213	Zhou <i>et al.</i> (2013)
<i>Sporothrix brasiliensis</i>	Human	Brazil	KC113214	Zhou <i>et al.</i> (2013)
<i>Sporothrix brasiliensis</i>	Human	Brazil	KF961142	Rodrigues <i>et al.</i> (2014)
<i>Sporothrix brunneoviolacea</i>	Endophyte in <i>Vitis vinifera</i>	USA	KC113235	Zhou <i>et al.</i> (2013)
<i>Sporothrix globosa</i>	Human	Spain	FN549904	Madrid <i>et al.</i> (2010a)
<i>Sporothrix globosa</i>	Human	Spain	KC113225	Zhou <i>et al.</i> (2013)
<i>Sporothrix globosa</i>	Human	Spain	KC113226	Zhou <i>et al.</i> (2013)
<i>Sporothrix humicola</i>	Soil	Netherlands	KC113233	Zhou <i>et al.</i> (2013)
<i>Sporothrix inflata</i>	Plant (<i>Pinus sylvestris</i>)	Poland	JX444611	Bilanski, P., Jankowiak, R. (unpublished)
<i>Sporothrix inflata</i>	Beetle (<i>Pinus sylvestris</i> , <i>Hylobius abietis</i>)	Poland	JX028590	Bilanski, P., Jankowiak, R. (unpublished)
<i>Sporothrix inflata</i>	Soil	Canada	AY495428	Aghayeva <i>et al.</i> (2005)
<i>Sporothrix inflata</i>	Soil	Chile	AY495429	Aghayeva <i>et al.</i> (2005)
<i>Sporothrix inflata</i>	Wheat-field soil	Germany	AY495426	Aghayeva <i>et al.</i> (2005)
<i>Sporothrix mexicana</i>	Environmental	Mexico	FN549906	Madrid <i>et al.</i> (2010a)
<i>Sporothrix mexicana</i>	Human	Brazil	KF961182	Rodrigues <i>et al.</i> (2014)
<i>Sporothrix pallida</i>	Feline (<i>Felis catus</i>)	Brazil	KF574458	Sasaki <i>et al.</i> (2014)
<i>Sporothrix pallida</i>	Sediment in water purification plant	Germany	EF127879	de Meyer <i>et al.</i> (2008)
<i>Sporothrix pallida</i>	Sporophore of <i>Stemonitis fusca</i>	Japan	EF127880	de Meyer <i>et al.</i> (2008)
<i>Sporothrix schenckii</i>	Growing in soil	South Africa	AF484471	de Beer <i>et al.</i> (2003)
<i>Sporothrix schenckii</i>	Growing in soil	South Africa	AF484472	de Beer <i>et al.</i> (2003)
<i>Sporothrix schenckii</i>	Human	Netherlands	AB128000	Kawasaki, A., Watanabe, S., Mochizuki, T. and Ishizaki, H. (published only in database 2003)
<i>Sporothrix schenckii</i>	Human	South Africa	AF484470	de Beer <i>et al.</i> (2003)
<i>Sporothrix schenckii</i>	Human	Italy	KC113216	Zhou <i>et al.</i> (2013)
<i>Sporothrix schenckii</i>	Human	France	KC113220	Zhou <i>et al.</i> (2013)
<i>Sporothrix schenckii</i>	Fungal (<i>Lentinula edodes</i>)	Japan	AB298704	Miyazaki, K., Tsuchiya, Y. and Okuda, T. (unpublished)
<i>Sporothrix schenckii</i>	Fungal (<i>Lentinula edodes</i>)	Japan	AB374289	Miyazaki, K., Tsuchiya, Y. and Okuda, T. (unpublished)
<i>Sporothrix schenckii</i>	Plant (<i>Rosa</i> sp.)	South Africa	AF484468	de Beer <i>et al.</i> (2003)
<i>Sporothrix schenckii</i>	Feline	Germany	IMT 15034	Weingart <i>et al.</i> (2010)
<i>Sporothrix schenckii</i> var. <i>luriei</i>	Human	South Africa	AB128012	Kawasaki, M., Watanabe, S. and Ishizaki, H. (published only in database (2003)
<i>Sporothrix</i> sp.	Plant (<i>Vitis</i> sp.)	New Zealand	EU770232	Weir, B.S. (unpublished)
<i>Sporothrix</i> sp.	Beetle (<i>Hylurgus ligniperda</i>)	USA	GU129986	Kim <i>et al.</i> (2011)
<i>Sporothrix</i> sp.	Plant (<i>Musa acuminata</i>) AAA Group cv. Cavendish	USA	GU377300	Tarnowski <i>et al.</i> (2010)
<i>Sporothrix</i> sp.	Beetle (<i>Orthotomicus erosus</i>)	USA	GU393352	Kim, S. and Harrington, T.C. (unpublished)
<i>Sporothrix</i> sp.	Soil	Spain	FN546959	Madrid <i>et al.</i> (2010a)
<i>Sporothrix</i> sp.	Soil	Spain	FN546961	Madrid <i>et al.</i> (2010a)
<i>Sporothrix</i> sp.	Soil	USA	FN546962	Madrid <i>et al.</i> (2010a)
<i>Sporothrix</i> sp.	Feline	Germany	IHIT 23589	This case report
<i>Sporothrix stylites</i>	Plant (wood pole)	South Africa	EF127881	de Meyer <i>et al.</i> (2008)
<i>Sporothrix stylites</i>	Plant (wood pole)	South Africa	EF127882	de Meyer <i>et al.</i> (2008)
<i>Sporothrix stylites</i>	Plant (wood pole)	South Africa	EF127883	de Meyer <i>et al.</i> (2008)
<i>Sporothrix stylites</i>	Plant (wood pole)	South Africa	EF127884	de Meyer <i>et al.</i> (2008)

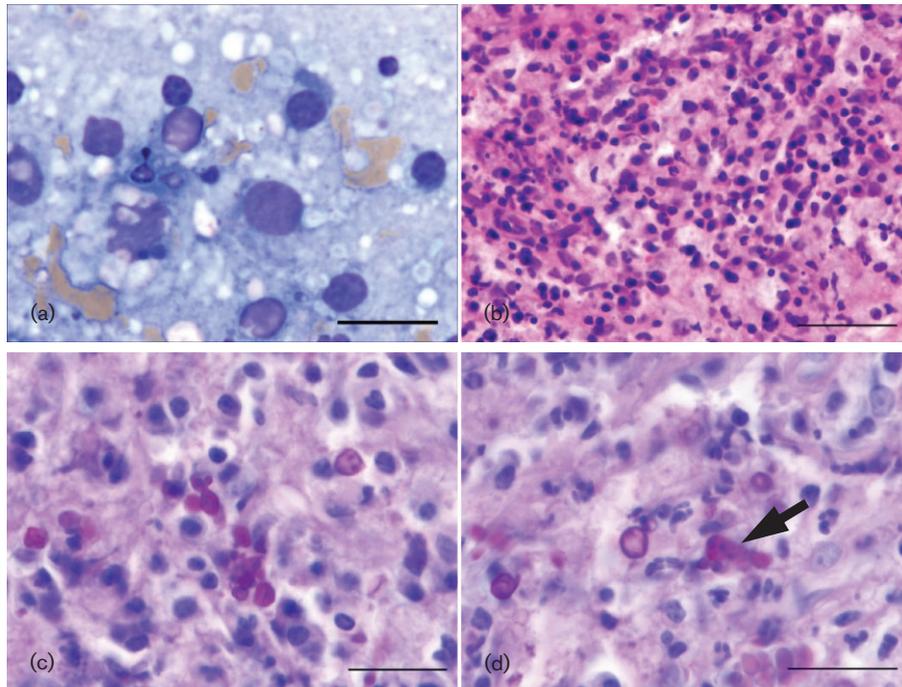


Fig. 1. (a) Yeast-like structures in a fine-needle aspiration smear of a nasal biopsy sample (May–Grünwald–Giemsa stain). (b) Nasal tissue sample showing chronic inflammation (H&E stain). (c) PAS-positive oval yeast-like organisms. (d) PAS-positive oval yeast-like organisms with small hyphae (arrow). Bars, 20 μm (a, c, d); 50 μm (b).

Outcome and follow-up

Control examinations were performed after 10 days, 15 days and 5 months. During this period, the thrombocytopenia persisted, with platelet counts ranging between 20000 and 55000 l^{-1} , whereas respiratory symptoms and nasal discharge improved remarkably after 3 weeks and disappeared completely after 6 weeks. The computed tomography scan revealed no further destruction of the nasal cavity.

Discussion

The cause of the respiratory symptoms of the cat was a chronic pyogranulomatous inflammation triggered by a mycotic process. Chronic nasal diseases in cats are quite common and – apart from a large number of cases where an aetiological agent cannot be found – are mostly related to neoplasia such as lymphoma or carcinoma, or to infectious agents such as feline herpesvirus 1, feline calicivirus, *Chlamydomphila* sp., *Mycobacterium* sp. and *Cryptococcus* sp. (Allen *et al.*, 1999; Demko & Cohn, 2007; Henderson *et al.*, 2004; Reed & Gunn-Moore, 2012). In this case, besides a few colonies of *Staphylococcus felis*, no other bacteria were found and therefore a bacterial event could be excluded. Although the cat has been examined thoroughly, a definite cause for the persisting thrombocytopenia could not be found. Cytological examination of a bone-marrow aspirate showed an active bone marrow with megakaryocytes present. Taking into account the report by Miller & Lunn (2007) who stated that exam-

inations of bone-marrow aspirates in a stage of severe isolated thrombocytopenia may not be of diagnostic or prognostic value, and due to the severity of the thrombocytopenia in the periphery, an immune-mediated process as a side effect of the mycosis had to be suspected.

Referring to the most prominent clinical signs (central impression of the nose, nasal discharge and a round mass near to the orbita), differential diagnoses such as neoplasia, mycobacteriosis, feline leprosy syndrome, nocardiosis and aspergillosis (Giordano *et al.*, 2010) were considered. However, the massive destruction of the nasal cavity together with the cytological and histopathological finding of yeast-like organisms quickly raised suspicion of a dimorphic fungus, especially with regard to the relatively high sensitivity of cytopathological examination as a diagnostic feature (Pereira *et al.*, 2011). Dimorphic fungi with a cutaneous manifestation in cats include *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis* and *S. schenckii* (Blondin *et al.*, 2007; Davies & Troy, 1996; Fischer *et al.*, 2013; Greene & Troy, 1995). Besides their variable endemic regions, these fungi differ in the appearance of their yeast-like phase in the cytological and histopathological examination: *Blastomyces dermatitidis* yeasts consist of a thick wall, a small capsule and broad-based budding figures, *Histoplasma capsulatum* is often present in the form of round yeasts with a small capsule within macrophages, organisms of *Coccidioides immitis* present as large spherules and yeasts of *S. schenckii* are pleomorphic (Valenciano &

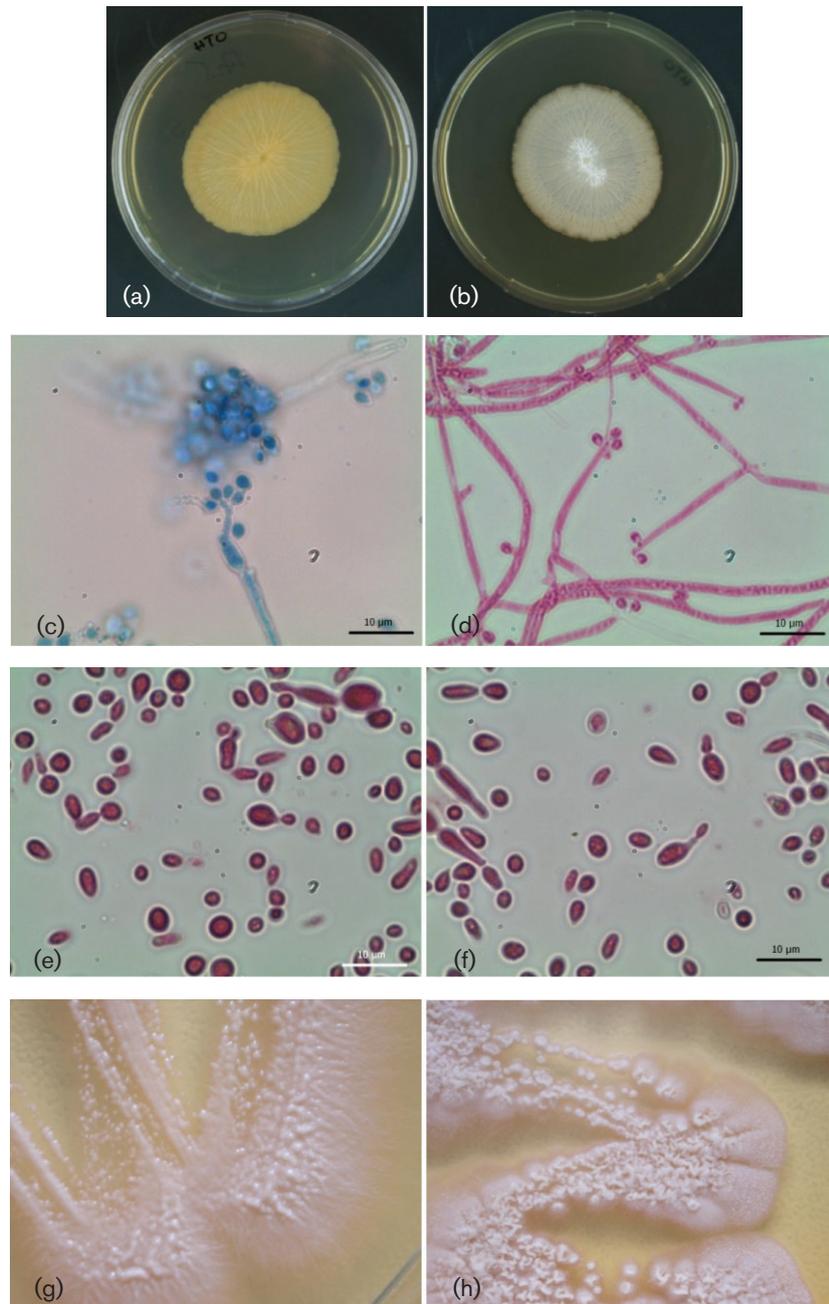


Fig. 2. (a, b) Back (a) and front (b) sides of a colony of *Sporothrix* sp. grown at 28 °C for 26 days on modified Kimmig agar. (c, d) Conidiogenous cells with conidia in clusters (c) or arising directly from hyphae of *Sporothrix* sp. (d). (e, f) Oval to pyriform yeast-like structures of *Sporothrix* sp. grown at 28 °C on modified Kimmig agar. (g) Smooth colony morphology of *Sporothrix* sp. grown at 28 °C for 19 days on modified Kimmig agar. (h) Wrinkled colony morphology of *Sporothrix* sp. grown at 37 °C for 12 days on modified Kimmig agar.

Cowell, 2014). Differences also exist concerning their morphological appearance in culture and their clinical manifestation. Besides *S. schenckii* and *Histoplasma capsulatum* var. *duboisii*, all other mentioned dimorphic fungi predominantly infect the lungs and cause systemic disease (Hogan *et al.*, 1996; Medleau *et al.*, 2007; Moore *et al.*, 2011).

Several phylogenetic studies have shown the heterogeneity of the genus *Sporothrix* and suggest that *S. schenckii* var. *schonckii* should not be the only member considered as the agent of sporotrichosis (Rodrigues *et al.*, 2013a, b; Zhou *et al.*, 2013). However, even though different species have been suggested, discussion of their level of virulence

was controversial as it seemed to vary between different species as well as within the same isolate according to its site of isolation (Arrillaga-Moncrieff *et al.*, 2009; Brito *et al.*, 2007; Howard & Orr, 1963). Melanin is considered a crucial virulence factor of *S. schenckii* isolates, although its expression varies among the species. For instance, Nobre *et al.* (2005) reported a difference between pigmented *S. schenckii* strains isolated from fixed cutaneous lesions versus systemic forms (Davis, 1915; Madrid *et al.*, 2010b; Nobre *et al.*, 2005). Comparing the morphology of the fungus isolated from this case with previously published cases of sporotrichosis in cats, its culture appearance of wrinkled to smooth colonies together with oval-shaped conidia growing in groups or in a sympodial manner is one of the main characteristics of the genus *Sporothrix* (de Hoog, 1995; Madrid *et al.*, 2012). However, as it remained amelanotic throughout the whole incubation process regardless of different culture conditions (Almeida-Paes *et al.*, 2009) and due to the lack of pigmentation in the histopathological examination, its classification outside the clinical clade of *S. schenckii sensu stricto* seems to be justified. The failure to produce melanin could be consistent with environmental strains like *S. pallida*. On the other hand, these strains are not usually responsible for major infections in immunocompetent patients, so it remains uncertain whether the strain in this case might have caused severe disease due to an immunocompromised status of the cat or, even though potentially related to environmental strains, it has a higher virulence in feline sporotrichosis than should be expected. Likewise, the simultaneous expression of yeast-like cells and hyphae might be due to the isolation site of the organism (Kwon-Chung, 1979). Clinical symptoms of feline sporotrichosis can vary and include cutaneous, lymphocutaneous and systemic forms (Schubach *et al.*, 2004). Cutaneous lesions in particular are described as multiple, often-ulcerating nodules and are associated with a high fungal load (dos Santos *et al.*, 2013; Miranda *et al.*, 2013). Assuming a cutaneous form of feline sporotrichosis in this case, inoculation of conidia after a trauma of the skin seems to be rational. However, as the clinical picture presented was atypical in the sense of just one non-ulcerating cutaneous nodule, the possibility of air-borne transmission of conidia with manifestation in the nasal cavity should be considered.

As far as phylogenetic classification is concerned, the short ITS sequence in combination with relatively low bootstrap values did not enable a more specific nomenclature of the isolated fungus at this point. However, further molecular investigation, such as through examination of the calmodulin gene, remains to be performed so that a phylogenetic analysis with sequences especially from cases of feline sporotrichosis can be conducted (Marimon *et al.*, 2006; Romeo *et al.*, 2011).

As this fungus has been isolated from a clinical case and therapy with itraconazole dissolved the symptoms, it most likely represented an atypical case of feline sporotrichosis caused by *Sporothrix* sp. related to the environmental clade. Epidemiological evidence is difficult due to the lack of comparable cases in Germany. To the best of our

knowledge, this case of feline sporotrichosis is the third described in Germany throughout the last 10 years and, although the infectious agent in the case in 2010 was described as a pigmented *S. schenckii* isolate, a zoonotic potential cannot be excluded (Weingart *et al.*, 2010). This case report therefore once again emphasizes the fact that uncommon agents should be taken into account in chronic dermal infections in cats.

Acknowledgements

We would like to thank our colleagues at the university clinic for performing the computed tomography scan, Dr Reinhard Weiss for providing his profound knowledge in discussing every aspect of this case, Dr Dagmar Rimek for performing the initial sequencing procedure and Dr Antina Lübke-Becker for providing the ITS sequence of their *Sporothrix* sp. isolate. The authors have no conflicts of interest to declare.

References

- Aghayeva, D. N., Wingfield, M. J., de Beer, Z. W. & Kirisits, T. (2004). Two new *Ophiostoma* species with *Sporothrix* anamorphs from Austria and Azerbaijan. *Mycologia* **96**, 866–878.
- Aghayeva, D. N., Wingfield, M. J., Kirisits, T. & Wingfield, B. D. (2005). *Ophiostoma dentifundum* sp. nov. from oak in Europe, characterized using molecular phylogenetic data and morphology. *Mycol Res* **109**, 1127–1136.
- Allen, H. S., Broussard, J. & Noone, K. (1999). Nasopharyngeal diseases in cats: a retrospective study of 53 cases (1991–1998). *J Am Anim Hosp Assoc* **35**, 457–461.
- Almeida-Paes, R., Frases, S., Fialho Monteiro, P. C., Gutierrez-Galhardo, M. C., Zancopé-Oliveira, R. M. & Nosanchuk, J. D. (2009). Growth conditions influence melanization of Brazilian clinical *Sporothrix schenckii* isolates. *Microbes Infect* **11**, 554–562.
- Arrillaga-Moncrieff, I., Capilla, J., Mayayo, E., Marimon, R., Marine, M., Genis, J., Cano, J. & Guarro, J. (2009). Different virulence levels of the species of *Sporothrix* in a murine model. *Clin Microbiol Infect* **15**, 651–655.
- Barros, M. B., de Almeida Paes, R. & Schubach, A. O. (2011). *Sporothrix schenckii* and sporotrichosis. *Clin Microbiol Rev* **24**, 633–654.
- Blondin, N., Baumgardner, D. J., Moore, G. E. & Glickman, L. T. (2007). Blastomycosis in indoor cats: suburban Chicago, Illinois, USA. *Mycopathologia* **163**, 59–66.
- Brilo, M. M. S., Conceição-Silva, F., Morgado, F. N., Raibolt, P. S., Schubach, A., Schubach, T. P., Schäffer, G. M. V. & Borba, C. M. (2007). Comparison of virulence of different *Sporothrix schenckii* clinical isolates using experimental murine model. *Med Mycol* **45**, 721–729.
- Davies, C. & Troy, G. C. (1996). Deep mycotic infections in cats. *J Am Anim Hosp Assoc* **32**, 380–391.
- Davis, D. J. (1915). Chromogenesis in cultures of *Sporotricha*. *J Infect Dis* **17**, 174–182.
- de Beer, Z. W., Harrington, T. C., Vismer, H. F., Wingfield, B. D. & Wingfield, M. J. (2003). Phylogeny of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia* **95**, 434–441.
- de Hoog, G. S. (editor) (1995). *Atlas of Clinical Fungi*. Reus, Spain: Centraalbureau voor Schimmelcultures.
- de Meyer, E. M., de Beer, Z. W., Summerbell, R. C., Moharram, A. M., de Hoog, G. S., Vismer, H. F. & Wingfield, M. J. (2008). Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia* **100**, 647–661.

- Demko, J. L. & Cohn, L. A. (2007). Chronic nasal discharge in cats: 75 cases (1993–2004). *J Am Vet Med Assoc* **230**, 1032–1037.
- dos Santos, I. B., Quintella, L. P., de Miranda, L. H. M., de Sousa Trotte, M. N., Schubach, T. M. P. & Tortelly, R. (2013). Atypical feline sporotrichosis resembling vaccine-induced sarcoma: clinical and histopathological aspects. *J Feline Med Surg* **15**, 517–519.
- Fischer, N. M., Favrot, C., Monod, M., Grest, P., Rech, K. & Wilhelm, S. (2013). A case in Europe of feline histoplasmosis apparently limited to the skin. *Vet Dermatol* **24**, 635–e158.
- Gardes, M. & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Mol Ecol* **2**, 113–118.
- Giordano, C., Gianella, P., Bo, S., Vercelli, A., Giudice, C., Della Santa, D., Tortorano, A. M., Peruccio, C. & Peano, A. (2010). Invasive mould infections of the naso-orbital region of cats: a case involving *Aspergillus fumigatus* and an aetiological review. *J Feline Med Surg* **12**, 714–723.
- Greene, R. T. & Troy, G. C. (1995). Coccidioidomycosis in 48 cats: a retrospective study (1984–1993). *J Vet Intern Med* **9**, 86–91.
- Henderson, S. M., Bradley, K., Day, M. J., Tasker, S., Caney, S. M. A., Hotston Moore, A. & Gruffydd-Jones, T. J. (2004). Investigation of nasal disease in the cat – a retrospective study of 77 cases. *J Feline Med Surg* **6**, 245–257.
- Hogan, L. H., Klein, B. S. & Levitz, S. M. (1996). Virulence factors of medically important fungi. *Clin Microbiol Rev* **9**, 469–488.
- Howard, D. H. & Orr, G. F. (1963). Comparison of strains of *Sporothrix schenckii* isolated from nature. *J Bacteriol* **85**, 816–821.
- Innis, M. A. (editor) (1990). *PCR protocols. A Guide to Methods and Applications*. San Diego: Academic Press.
- Kim, S., Harrington, T. C., Lee, J. C. & Seybold, S. J. (2011). *Leptographium tereforme* sp. nov. and other Ophiostomatales isolated from the root-feeding bark beetle *Hylurgus ligniperda* in California. *Mycologia* **103**, 152–163.
- Kwon-Chung, K. J. (1979). Comparison of isolates of *Sporothrix schenckii* obtained from fixed cutaneous lesions with isolates from other types of lesions. *J Infect Dis* **139**, 424–431.
- Madrid, H., Gené, J., Cano, J., Silvera, C. & Guarro, J. (2010a). *Sporothrix brunneoviolacea* and *Sporothrix dimorphospora*, two new members of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia* **102**, 1193–1203.
- Madrid, I. M., Xavier, M. O., Mattei, A. S., Fernandes, C. G., Guim, T. N., Santin, R., Schuch, L. F. D., Nobre, M. O. & Araújo Meireles, M. C. (2010b). Role of melanin in the pathogenesis of cutaneous sporotrichosis. *Microbes Infect* **12**, 162–165.
- Madrid, I. M., Mattei, A. S., Fernandes, C. G., Nobre, M. O. & Meireles, M. C. A. (2012). Epidemiological findings and laboratory evaluation of sporotrichosis: a description of 103 cases in cats and dogs in southern Brazil. *Mycopathologia* **173**, 265–273.
- Marimon, R., Gené, J., Cano, J., Trilles, L., Dos Santos Lazéria, M. & Guarro, J. (2006). Molecular phylogeny of *Sporothrix schenckii*. *J Clin Microbiol* **44**, 3251–3256.
- Marimon, R., Cano, J., Gené, J., Sutton, D. A., Kawasaki, M. & Guarro, J. (2007). *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. *J Clin Microbiol* **45**, 3198–3206.
- Medleau, L., Hnilica, K. A. & Coyner, K. S. (2007). *Dermatologie in der Kleintierpraxis: Atlas und Therapiehandbuch*. Munich, Germany: Elsevier Urban & Fischer.
- Miller, M. D. & Lunn, K. F. (2007). Diagnostic use of cytologic examination of bone marrow from dogs with thrombocytopenia: 58 cases (1994–2004). *J Am Vet Med Assoc* **231**, 1540–1544.
- Miranda, L. H. M., Conceição-Silva, F., Quintella, L. P., Kuraiem, B. P., Pereira, S. A. & Schubach, T. M. P. (2013). Feline sporotrichosis: histopathological profile of cutaneous lesions and their correlation with clinical presentation. *Comp Immunol Microbiol Infect Dis* **36**, 425–432.
- Moore, D., Robson, G. D. & Trinci, A. P. J. (2011). *21st Century Guidebook to fungi*. Cambridge, NY: Cambridge University Press.
- Nobre, M. O., Antunes, T. A., de Faria, R. O., Cleff, M. B., Fernandes, C. G., Muschner, A. C., Meireles, M. C. & Ferreira, L. (2005). Differences in virulence between isolates of feline sporotrichosis. *Mycopathologia* **160**, 43–49.
- Pereira, S. A., Menezes, R. C., Gremião, I. D. F., Silva, J. N., Honse, C. O., Figueiredo, F. B., da Silva, D. T., Braha Kitada, A. A., dos Reis, E. G. & Schubach, T. M. P. (2011). Sensitivity of cytopathological examination in the diagnosis of feline sporotrichosis. *J Feline Med Surg* **13**, 220–223.
- Reed, N. & Gunn-Moore, D. (2012). Nasopharyngeal disease in cats: 1. Diagnostic investigation. *J Feline Med Surg* **14**, 306–315.
- Rodrigues, A. M., de Melo Teixeira, M., de Hoog, G. S., Schubach, T. M., Pereira, S. A., Fernandes, G. F., Bezerra, L. M., Felipe, M. S. & de Camargo, Z. P. (2013a). Phylogenetic analysis reveals a high prevalence of *Sporothrix brasiliensis* in feline sporotrichosis outbreaks. *PLoS Negl Trop Dis* **7**, e2281.
- Rodrigues, A. M., de Hoog, G. S. & de Camargo, Z. P. (2013b). Emergence of pathogenicity in the *Sporothrix schenckii* complex. *Med Mycol* **51**, 405–412.
- Rodrigues, A. M., de Hoog, G. S. & de Camargo, Z. P. (2014). Genotyping species of the *Sporothrix schenckii* complex by PCR-RFLP of calmodulin. *Diagn Microbiol Infect Dis* **78**, 383–387.
- Romeo, O., Scordino, F. & Criseo, G. (2011). New insight into molecular phylogeny and epidemiology of *Sporothrix schenckii* species complex based on calmodulin-encoding gene analysis of Italian isolates. *Mycopathologia* **172**, 179–186.
- Sasaki, A. A., Fernandes, G. F., Rodrigues, A. M., Lima, F. M., Marini, M. M., Feitosa, L. d. S., de Melo Teixeira, M., Felipe, M. S. S., da Silveira, J. F. & de Camargo, Z. P. (2014). Chromosomal polymorphism in the *Sporothrix schenckii* complex. *PLoS One* **9**, e86819.
- Schubach, T. M., Schubach, A., Okamoto, T., Barros, M. B., Figueiredo, F. B., Cuzzi, T., Fialho-Monteiro, P. C., Reis, R. S., Perez, M. A. & Wanke, B. (2004). Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998–2001). *J Am Vet Med Assoc* **224**, 1623–1629.
- Schubach, A., Schubach, T. M., Barros, M. B. & Wanke, B. (2005). Cat-transmitted sporotrichosis, Rio de Janeiro, Brazil. *Emerg Infect Dis* **11**, 1952–1954.
- Talbot, N. J. (editor) (2001). *Molecular and Cellular Biology of Filamentous Fungi. A Practical Approach*. Oxford: Oxford University Press.
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.
- Valenciano, A. C. & Cowell, R. L. (editors) (2014). *Cowell and Tyler's Diagnostic Cytology and Hematology of the Dog and Cat*, 4th edn. St Louis: Elsevier.
- Weingart, C., Lübke-Becker, A. & Kohn, B. (2010). [*Sporothrix schenckii* infection in a cat]. *Berl Munch Tierarztl Wochenschr* **123**, 125–129.
- Welsh, R. D. (2003). Sporotrichosis. *J Am Vet Med Assoc* **223**, 1123–1126.
- Zhou, X., Rodrigues, A. M., Feng, P. & de Hoog, G. S. (2013). Global ITS diversity in the *Sporothrix schenckii* complex. *Fungal Divers* **66**, 153–165.