

Eosinophilic plasmacytic conjunctivitis concurrent with gingival fistula caused by *Schizophyllum commune* in a captive cheetah (*Acinonyx jubatus*)



Madoka Yoshizawa^{a,b,1}, Shinpei Kawai^{c,1}, Yoshiko Torii^{a,d}, Kaori Ota^a, Kiyoshi Tasaka^a, Kazuko Nishimura^e, Chieko Fujii^a, Nobuyuki Kanemaki^{c,*}

^a Tama Zoological Park, 7-1-1, Hodokubo, Hino-shi, Tokyo 191-0042, Japan

^b Tokyo Sea Life Park, 6-2-3, Rinkaicho, Edogawa-ku, Tokyo 134-8587, Japan

^c Laboratory of Small Animal Clinics, Veterinary Teaching Hospital, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagami-hara, Kanagawa 252-5201, Japan

^d Laboratory of Theriogenology, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

^e Department of Microbiology, Dokkyo Medical University School of Medicine, 880 Kitakobayashi, Mibu-machi, Shimotsuga-gun, Tochigi 321-0293, Japan

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ABSTRACT

We describe for the first time the diagnosis of *Schizophyllum commune* infection in a captive cheetah. Eosinophilic plasmacytic conjunctivitis was detected histopathologically in a biopsy specimen. Both a second surgical specimen and drainage fluid from a gingival mass and fistula contained fungal hyphae in giant cells with granulomatous inflammation. Allergic *S. commune* mycosis was suspected at this point. A monokaryotic isolate was characterized morphologically, and then identified genetically. Treatment with itraconazole and pimelic acid was effective.

1. Introduction

The basidiomycete *Schizophyllum commune*, known as “split gill”, is a common species of mushroom. It can be isolated worldwide and grows naturally on the rotting wood, fallen branches, and timber of deciduous trees. It has been used as a tractable model in mushroom research, and as an efficient resource for biological engineering [1]; however, reportedly it is occasionally responsible for opportunistic infections in humans and animals [2–8].

The incidence of *S. commune* infections is gradually increasing in humans, and it has occurred in Japan [9,10]. The characteristic associated clinical features include eosinophilic inflammation, allergic bronchopulmonary mycosis (ABPM) [2,11], allergic fungal rhinosinusitis (AFRS) [12] and mucoid impaction of the bronchi (MIB) [3,11]. Fungal keratitis has also recently been described [13,14], and invasion of the lung, brain, and palate have been reported in both immunocompromised and immunocompetent patients [15].

There are only four reported cases of the isolation of *S. commune* in animals [5–8]. Two were canine cases involving osteomyelitis [6,7], one was a canine case involving subcutaneous granuloma and pneumonia [5], and recently lethal systemic invasion of the eyes, lung, heart, and lymph node was described in a harbor seal [8]. There are some reports of good responses to antifungal drugs and/or

corticosteroids in humans [9,10,12,14] and dogs [7]. However, it is very difficult to diagnose an *S. commune* infection because the clinical signs of ABPM are nonspecific; thus, differentiating the condition from other allergic diseases is problematic [16]. Isolation of *S. commune* from sputum is uncommon, and even when it is detected it is often regarded as a contaminant in humans [1]. In animals, the diagnosis of *S. commune* infection requires specific cytological and/or pathological findings as well as its isolation directly from infected tissues [5–7]. We report here our efforts to diagnose and treat the first known case of allergic *S. commune* infection in a cheetah (*Acinonyx jubatus*).

2. Case

A 5-year-old female cheetah with a body weight (BW) of 40 kg that was born in captivity and maintained in good health exhibited signs of returning to estrus. The animal had been born at Tama Zoological Park in Tokyo, Japan and had been vaccinated for feline herpes virus 1 (FHV1), feline calicivirus (FCV), and feline panleukopenia virus (FPV) (Feline-CPR-NA, Kyoto Biken Laboratories, Kyoto, Japan) every year, and given ivermectin and/or pyrantel pamoate every 2 months for the prophylaxis of heart worms and internal parasites.

* Corresponding author.

E-mail address: kanemaki@azabu-u.ac.jp (N. Kanemaki).

¹ These authors equally contributed in this report.

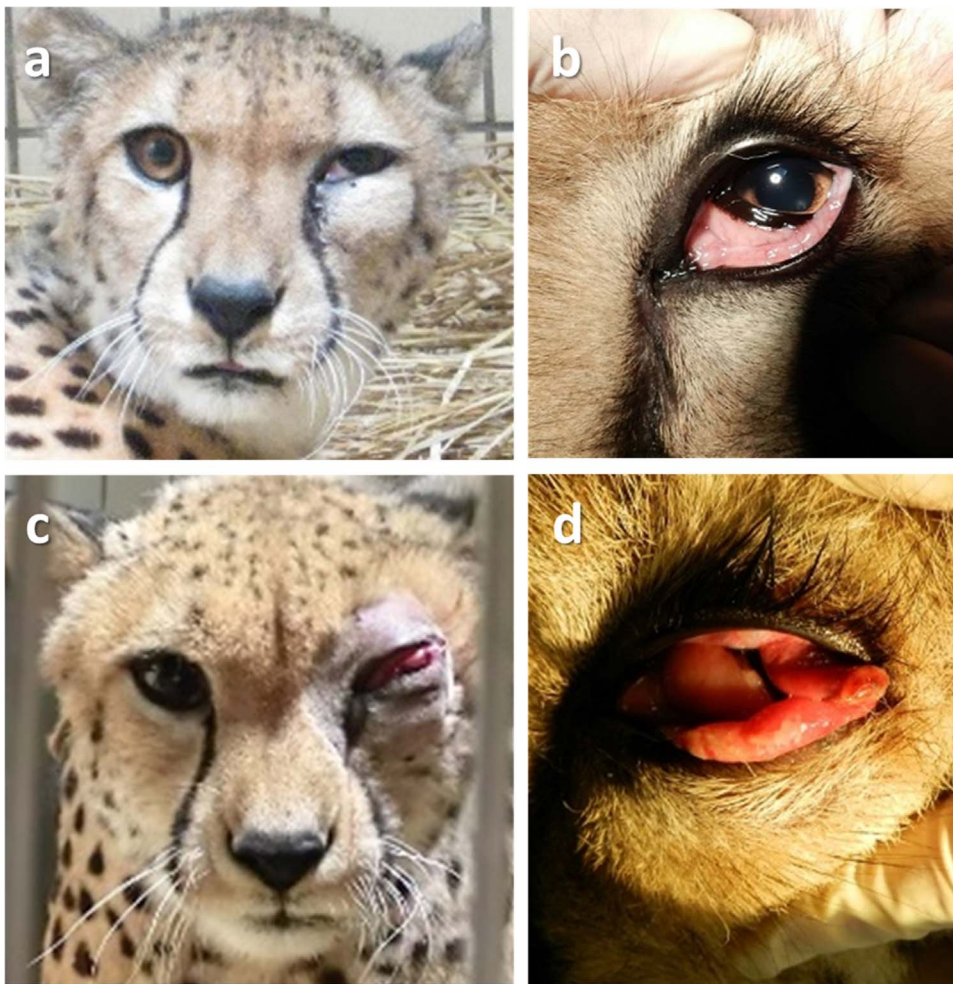


Fig. 1. The appearance of the head and left eye before the initiation of treatment (a) Day 4; (b) Day 3; (c) Day 15; (d) Day 14.

2.1. First differential diagnosis

A zookeeper initially noticed swelling of the left inferior palpebra, which gradually grew bigger over the subsequent 40 days. The veterinarians of the Wild Animal Clinic at Tama Zoological Park then examined the cheetah (day 0). Under general anesthesia, a general physical examination was conducted and a more thorough examination of the eyes, oral cavity, and surrounding areas was performed. No abnormalities were observed, and there was no chemosis of the conjunctiva (Fig. 1a and b). A bacterial culture (Kotobiken Medical Laboratories, Tokyo, Japan), FCV antigen test, and FHV1 and *Chlamydia felis* antibody titers, were tested for the differential diagnosis of these infections; all of these tests were negative.

A blood sample was collected for a complete blood cell count and serum biochemistry, and eosinophilia ($4.13 \times 10^9/L$ vs. reference ranges [17] of 0.09–2.80) was detected (Table 1). Oral antibiotics were administered, but the enlarged conjunctiva of the left inferior palpebra continued to increase in size. The redness and inflammation spread to the superior palpebra and nictitating membrane (Fig. 1c and d).

On day 14, the animal was brought to the Wild Animal Clinic for a second, detailed examination under anesthesia. No abnormalities of the eyeball, cornea, skin surface around the left eye, or oral cavity were detected during the physical examination. A swab sample was obtained from the left conjunctiva. Bacterial and fungal culture tests were conducted again (Kotobiken Medical Laboratories), but the results were negative. An incisional biopsy specimen was obtained from the naked conjunctiva at the left inferior palpebra. Eosinophilic plasmacytic inflammation was diagnosed via histopathology performed at a commercial laboratory (IDEXX Japan, Tokyo, Japan) (Fig. 2a and b). Based

Table 1

Total and differential white blood cell counts during the treatment period.

	Day 0	Day 14	Day 98	Reference range [17]
White blood cell count ($\times 10^9/L$)	17.2	12.52	16.51	(6.9–17.6)
Differential count				
Neutrophils-segmented ($\times 10^9/L$)	9.63	7.88	10.56	(4.9–15.7)
Neutrophils-bands ($\times 10^9/L$)	1.03	0.88	1.49	(not described)
Lymphocytes ($\times 10^9/L$)	1.89	2.13	1.32	(0.9–3.1)
Monocytes ($\times 10^9/L$)	0.52	0.13	0.17	(0.07–0.64)
Eosinophils ($\times 10^9/L$)	4.13	1.5	2.97	(0.09–2.80)
Basophils ($\times 10^9/L$)	0	0	0	(not described)

on the results of histopathology and the fact that no infectious agents were detected, a tentative diagnosis of feline eosinophilic granuloma complex was made. On day 27, once-daily oral administration of prednisolone (Shionogi, Osaka, Japan) 1 mg/kg BW was initiated.

2.2. Treatment and outcome after the first diagnosis

On day 30, the rate of progression of the enlargement and inflammation in the palpebra was reduced. The excessive lacrimation was reduced and the tear color became clear. Prednisolone was then tapered, and twice-daily oral administration of cyclosporine 2.5 mg/kg BW (Ciclosporin Capsule, Nichi-Iko, Toyama, Japan) was initiated on day 38. On day 53, pruritus around the left eye and sneezing appeared. The progression of the enlargement and inflammation in the palpebra

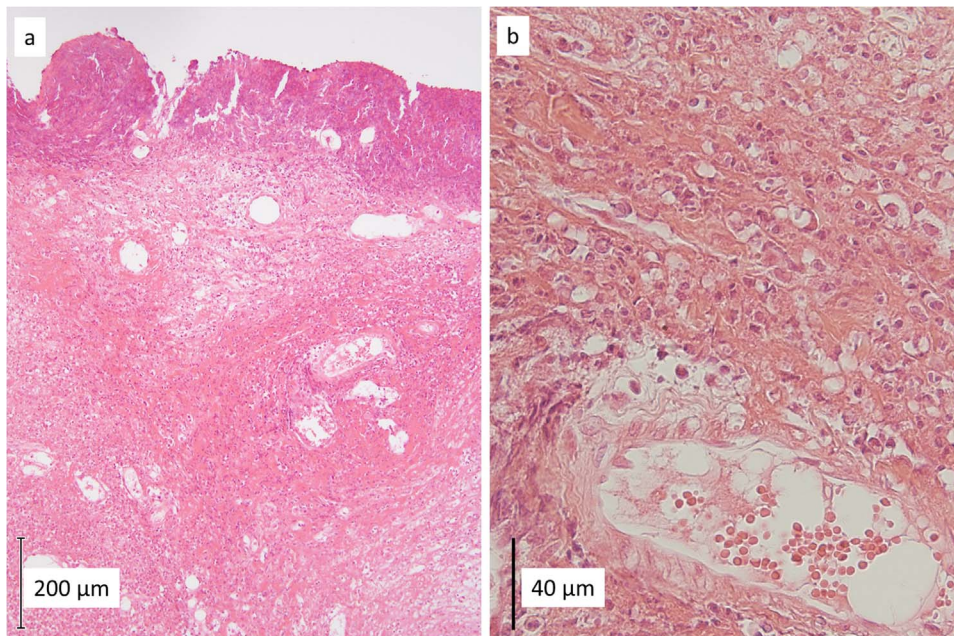


Fig. 2. Histopathological observations of surgical biopsy specimens from the conjunctiva (hematoxylin and eosin stain) (a) ulceration of the conjunctival epithelium, inflammatory vasodilation and edema, and infiltration of inflammatory cells at the sub-conjunctiva; (b) the inflammatory cells comprised numerous polymorphonuclear cells (eosinophils and neutrophils) and a few mononuclear cells.

re-commenced and the subcutaneous enlargement extended through the left inferior palpebra to the malar region. On day 60, the administration of cyclosporine and prednisolone was changed to Celestamine (betamethasone 0.25 mg and d-chlorpheniramine maleate 2 mg; Takata Pharmaceuticals, Saitama, Japan), 2 tablets, three times/day. We administered antibiotics as an adjunct therapy, testing different types for efficacy approximately every 14 days. The clinical response to the immunosuppressant drugs was poor however, and purulent nasal discharge developed.

2.3. Re-evaluation of the differential diagnosis

A second opinion was sought from the Department of Ophthalmology of the Animal Teaching Hospital of Azabu University, Kanagawa, Japan. On day 67, the following differential diagnoses were re-evaluated: fungal infection, cutaneous and ocular larva migrans, and malignant tumors. Both direct and saline-saturated fecal flotation examinations were negative. Fungal culture tests were performed again using a dermatophyte test medium (DTM) (Kyoritsu-seiyaku, Tokyo, Japan) at Azabu University. A white, felt-like mycelial colony grew on the DTM without changing color during 2 weeks of culturing. The fungus was deemed to be a dermatophyte based on its colony formation, but could not be identified more specifically microscopically. After the positive fungal culture test, glucocorticoid therapy was gradually tapered. We initiated oral administration of once-daily itraconazole (Sawai Medical, Osaka, Japan) at 5 mg/kg BW, and twice-daily clindamycin hydrochloride (Dalacin Capsules, Pfizer, Tokyo, Japan) at 5 mg/kg BW. Pimaricin drops (Pimaricin Senju, Senju Seiyaku, Osaka, Japan) were administered into the left eye three times per day. Aggravation of the lesion remained apparent, and mucopurulent discharge from the left palpebral fissure was observed on day 73 (Fig. 3a). The mucopurulent discharge stopped spontaneously on day 89, at which point the enlargement of the lesion began to reduce rapidly (Fig. 3b).

On day 95, a veterinarian noticed a gingival mass located laterally to a left upper molar (Fig. 3c). Another detailed examination was performed under general anesthesia on day 98. The gingival mass was 1.5 cm in diameter and originated from the oral vestibule, and there was a fistulous tract at the root of left upper molar (Fig. 3d). The tract terminated at a large subcutaneous abscess in the malar region. A surgical biopsy of the gingival mass was taken, and a 10% povidone-

iodine solution diluted 15-fold with saline was injected through a catheter into the fistulous tract. The subcutaneous abscess was washed and then injected with 3 ml of pimaricin.

Cytological examination of the drainage fluid from the fistula revealed pyogranulomatous inflammation with many macrophages and giant cells phagocytosing fungal hyphae (Fig. 4a). The surgical specimen was submitted to a commercial laboratory (IDEXX). A fungal granuloma was diagnosed (Fig. 4b and c), and periodic acid-Schiff staining revealed the presence of fungal hyphae within the multinucleated giant cells. Fungal culturing on DTM and Sabouraud dextrose agar resulted in the growth of the same white, felt-like colony observed previously (Fig. 4d).

2.4. Identification of *S. commune*

To identify the species of the clinical isolate, molecular analysis of the D2 region of the large-subunit (28 S) ribosomal RNA gene was performed. Genomic DNA was extracted using a MasterPure Yeast DNA purification kit (Epicenter, Osaka, Japan). Polymerase chain reaction amplification of the target gene was performed at a commercial laboratory (Fasmac, Kanagawa, Japan). The genome sequence identified was compared with previously reported sequences via MicroSEQ® ID Fungal Gene Library v2013, and was a 100% match with *S. commune* (number 579.83) in the Centraalbureau voor Schimmelcultures database.

Macro- and microscopic observations strongly suggested that the cultured isolate consisted of *S. commune* monokaryotic mycelium. The colony grew on plated agar, and exhibited fine filaments. The fungal plaque on the surface of the agar was moderately hard, but weaker than dikaryotic mycelium. It was composed of septate, branched, and hyaline thin-walled fine and thick-walled heavy hyphae. There was no clump-connection structure on the septa. Spine-like structures and/or tubercle structures were observed on the hyphae (Fig. 4e).

2.5. Outcome and follow-up after the second diagnosis

The clinical response to antifungal drugs was good, but anorexia was observed on approximately day 85, and lethargy developed approximately 2 weeks thereafter. Alanine aminotransferase had increased from 0.99 μ kat/L on day 14 to 9.77 μ kat/L on day 98 (vs. reference ranges of 0.82–3.31 derived from the literature [17]). The

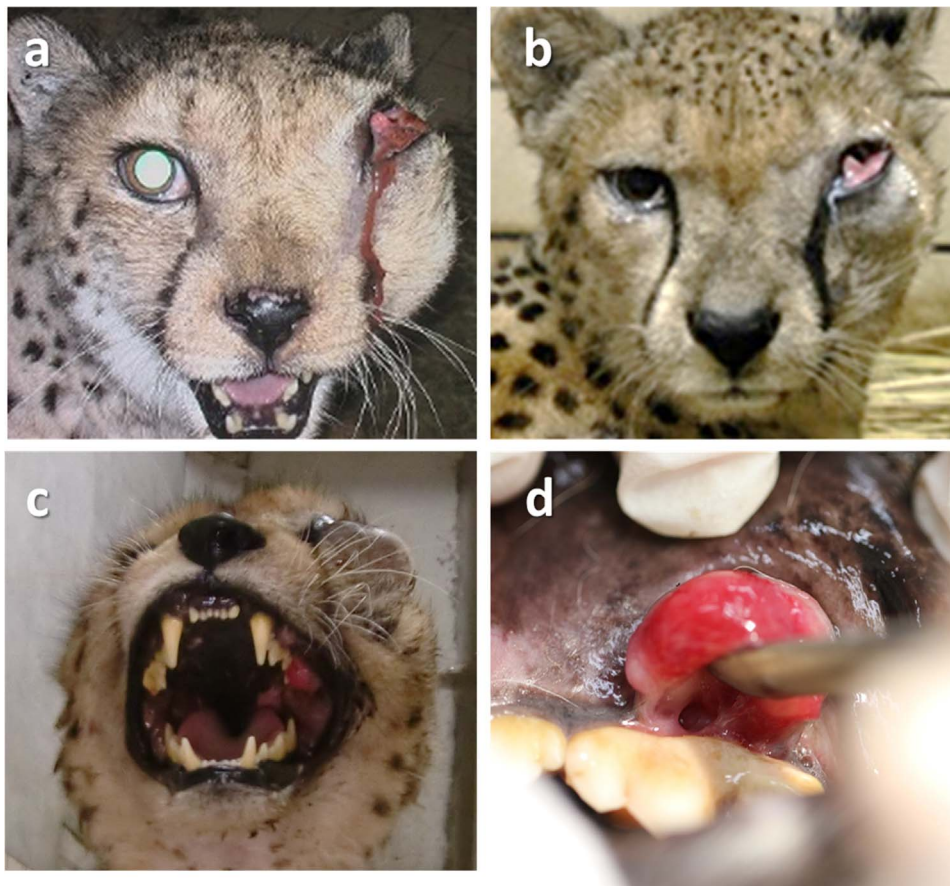


Fig. 3. Appearance after initiating treatment for *S. commune* infection. The appearance of the head and left eye on days 73 (a), 99 (b), and 95 (c), and the gingival mass and fistula on day 98 (d).

elevation of liver enzymes suggested an adverse reaction to itraconazole; thus, withdrawal was initiated with a stepwise dose reduction every other week, and once-daily administration of ursodeoxycholic acid 10 mg/kg BW (Urso, Mitsubishi Tanabe Pharma, Osaka, Japan) was begun. Because the anorexia continued during the withdrawal period, the itraconazole was terminated on day 119. Within 2 weeks of instituting these changes, the cheetah's appetite recovered and it returned to consuming a normal diet.

To confirm clinical resolution, a fourth examination under general anesthesia was performed on day 147. The gingival fistula was closed, but the swelling was still palpable. Amphotericin B solution was injected directly into the left inferior palpebra. A sparse aspirate was obtained from the same lesion and cultured on DTM, resulting in the growth of a white, felt-like colony over the subsequent 4 weeks. The swelling of the left inferior palpebra gradually reduced, and near complete recovery was apparent on day 192 (Fig. 5).

3. Discussion

To the best of our knowledge, this is the first report of *S. commune* infection in a cheetah. Furthermore, we believe that it is the first report of such an infection in any species within the *Felidae* family. Osteomyelitis and lethal systemic dissemination involving respiratory organs have been reported previously in the context of animal *S. commune* infections [5–8], but none of those reports described eosinophilic inflammation, which is a common finding in humans [2,3,9–12]. The ulcerative lesions on the hard palate and the persistent eosinophilia detected during the treatment period indicate that the allergic and invasive pathogenicity of *S. commune* may have been severe in the present case.

After the diagnosis of an *S. commune* infection, responses to antifungal drugs such as itraconazole and pimaficin (natamycin) are

reportedly good in humans and animals [7,9,10,14]; the present case also responded well to this treatment regimen, despite the animal's reaction to itraconazole. In the present case, signs of a return to estrus were apparent, and we repeatedly observed the cheetah rubbing her face against deadwood. An immunocompromised condition induced by a hormone imbalance may have contributed to the etiology of *S. commune* infection in the present case [15].

Toyotome et al. [16] recently identified a major antigenic protein, Sch c 1, in the supernatant of *S. commune* cultures, and guidelines for the diagnosis of *S. commune*-associated sinobronchial allergic mycosis (SAM) utilizing Sch c 1-specific IgE have been proposed in Japan [18]. The fundamental symptoms are eosinophilic MIB and/or eosinophilic mucin involving multiple sinuses. The major diagnostic criteria are a positive fungal culture of *S. commune* from bronchial specimens or sinus contents and positive results for *S. commune*-specific IgE and/or IgG. The supplemental findings are elevated peripheral eosinophils and/or total serum IgE levels, and radiographic evidence of ABPM and/or AFRS. Notably, while we could not measure *S. commune*-specific IgE in the present case, the eosinophilic inflammation of the conjunctiva, the peripheral blood eosinophilia, and the isolation of *S. commune* from the fistula satisfied the above criteria for a diagnosis of SAM.

A. jubatus has been red-listed by the International Union for the Conservation of Nature and National Resources [19]. One of the reasons why wild animal zoos around the world keep cheetahs is to promote the conservation of species. The case report presented herein will contribute the accumulation of veterinary knowledge relating to cheetahs that can be applied to those in captivity, and may also contribute to veterinary care of the *Felidae* family in general.

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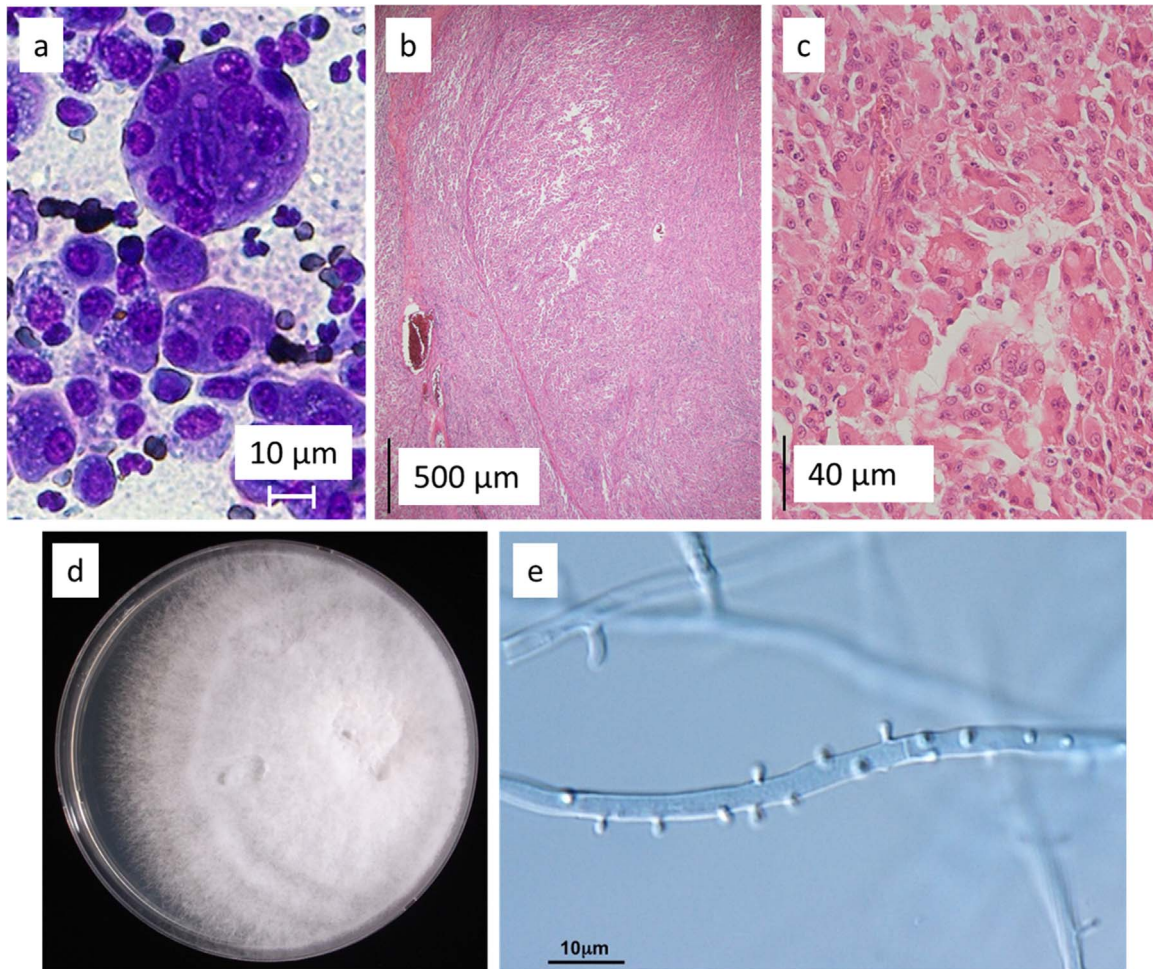


Fig. 4. (a) Photomicrograph of the drainage fluid from the gingival fistula during cytological analysis. Pyogranulomatous inflammation with many macrophages and giant cells phagocytosing fungal hyphae were evident. Wright Giemsa staining. (b, c) Histopathological results of surgical specimens from the gingival mass (hematoxylin and eosin stain). The mass was composed of many large lobules of granuloma that was composed of large epithelioid macrophages. Giant cells were observed predominantly at the center of lobules. At higher magnification, the giant cells and large macrophages demonstrated phagocytosed fungal hyphae within their cytoplasm. (d, e) Characteristics of *Schizophyllum commune* cultures. *S. commune* colony cultured from the gingival fistula drainage fluid. The colony was cultivated on Sabouraud dextrose agar for 7 days at 37° C, then 4 days at 25° C. Photomicrograph of *S. commune* hyphae (lactophenol cotton blue stain). There was no clump-connection structure on the septa (arrow). Spine-like structures and tubercle structures were observed in parts of the hyphae.

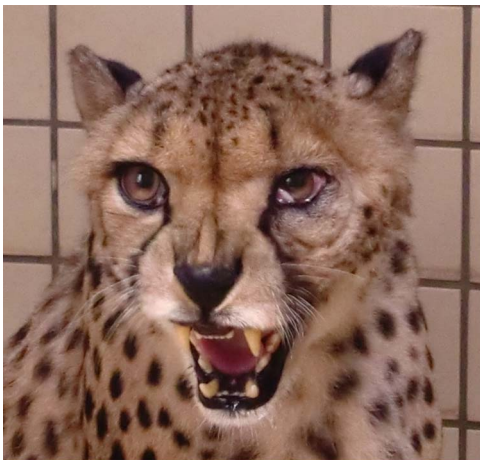


Fig. 5. Appearance of the head and left eye on day 160. This image was taken approximately one month before clinical resolution was considered nearly complete.

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Conflicts of interest

There are no conflicts of interest to declare.

Ethical form

The care and treatment of the animal in this case report was performed according to the guidelines of the Japanese Association of Zoos and Aquariums.

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