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# Dermatophytosis caused by *Trichophyton benhamiae* in a sea lion. First report

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ABSTRACT

Fungal infections in marine animals, particularly pinnipeds, have seen a notable increase, often linked to compromised immune systems in captive environments. *Trichophyton* species, while common in terrestrial mammals, have sporadically caused dermatophytosis in pinnipeds. A South American sea lion (*Otaria byronia*) presented with *Trichophyton benhamiae* infection, marking the first such case in this species. Effective treatment combined oral terbinafine with topical ozonized oil, supported by silymarin for liver protection. Accurate fungal identification and sensitivity testing were key to the successful management and recovery of the patient.

## 1. Introduction

Over recent decades, the epidemiology of fungal infections in marine animals has undergone a significant shift [1]. The incidence of these infections has markedly increased, particularly among animals under human care in tropical and subtropical climates [2]. Several reports have identified *Candida* spp., *Fusarium* sp., *Malassezia pachydermatis*, and *Rhodotorula mucilaginosa* as some of the fungal agents associated with superficial mycoses in different species of pinnipeds [3–6]. Immunosuppression is considered the most important predisposing factor [1], given that these captive animals are frequently exposed to disinfectants in the water, increased use of corticosteroids, antibiotics, malnutrition, and stress-related management factors [4,7,8].

Dermatophytosis, or ringworm, is a rarely reported mycosis in pinnipeds [8,9]. In this context, *Nannizzia gypsea*, formerly known as *Microsporum gypseum* [10], *Trichophyton mentagrophytes* and *T. rubrum* have been isolated from skin lesions in captive seals and sea lions [8,9,11,12], whit an affinity for keratinized tissues, the most frequent clinical signs include non-pruritic focal or multifocal alopecia, which may exhibit varying degrees of erythema and/or ulceration [13]. Although this type of mycosis rarely becomes systemic or fatal, timely detection and accurate identification of the causative agent are essential. Molecular techniques have become a useful tool for precise fungal pathogen identification, facilitating specific antifungal treatment and preventing recurrences after discharge.

We present the first case of dermatophytosis due by *Trichophyton benhamiae* in a South American Sea Lion *Otaria byronia* (de Blainville, 1820) (also known as *O. flavescens* Shaw, 1800), one of the most exhibited species in zoos, aquariums, and marine parks worldwide [14].

## 2. Case

A 13-year-old female South American Sea Lion *Otaria byronia*; was clinically evaluated by the specialist veterinarian, for alopecia and mildly erythematous and scaly lesions (day 0). One lesion, approximately 10 cm in diameter, was located on the animal's back, while smaller lesions were present on one sides of the body (Fig. 1A and B). The lesions had been developing for approximately one month. The patient resides in a marine park, where it is exhibited daily alongside two birds, with which it maintains indirect contact. The enclosure consists of two circular pools, each with a capacity of 30 $m^3$ each, with a closed water recirculation system, and a resting area covered with a

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mesh or roofing. Twice o three times a week, the enclosure undergoes a process of emptying, washing, and disinfection with chlorine. The same day, hair, and scale samples from the periphery of the lesions were collected by the veterinarian and transported in a sterile container. Analyses were performed at the Clinical Microbiology and Microbiome Laboratory of Andrés Bello University. A direct microscopic examination of the hair and scales was conducted using 10 % lactophenol. At the same time, the sample was plated on Sabouraud dextrose agar with chloramphenicol and cycloheximide and incubated at 25 °C for 28 days.

The macro- and microscopic characteristics of the colony allowed for the identification of *Trichophyton* spp. (Fig. 2) from a new colony DNA extraction was performed with the ZR Fungal/Bacterial DNA MiniPrep kit (ZYMO Research, Irvine, CA, USA). DNA was quantified using a Nanodrop 2000c nanospectrophotometer (ThermoScientific, Waltham, MA, USA). 10 ng of the extracted DNA were used for PCR amplification of the nuclear ribosomal internal transcribed spacer (ITS) regions 4 and 5, using the primers 5′-TCCTCCGCTTATTGATATGC-3′ and 5′-GGAAGTAAAAGTCGTAACAAGG-3′ and, on the other hand, for the calmodulin gene, using the primers Cmd5 5′-CCGAGTACAAGGARGCCTTC-3′ and Cmd6 5′-CCGATRGAGGTCATRACGTGG-3′ [15]. The sequences obtained from the PCR-amplified regions were compared with the sequences published in GenBank using the Basic Local Alignment Search Tool (BLAST). A percentage of identity and coverage >99 % were used as criteria to confirm the correct identification of the fungal species *Trichophyton benhamiae,* available in the GenBank database with the access codes PQ144852 and PQ150387 for ITS and calmodulin, respectively.

In parallel, the sensitivity of the strain to the antifungals griseofulvin, ketoconazole, itraconazole, posaconazole and terbinafine was determined according to the guidelines outlined in document M-38-A2 (CLSI, 2008). Sensitivity tests showed high minimal inhibitory concentration (MIC) values for the evaluated azoles (>1 μg/mL). Griseofulvin, on the other hand, showed an MIC of 0.05 μg/mL, but was excluded due to it hepatotoxicity. Consequently, on day +32 the patient begin was treated with a combination of oral (P.O.) terbinafine at 2.5 mg/kg/day (Finex, Saval Laboratory, Santiago, Chile), and a topical treatment with ozonized oil, administered once daily for 3 months (Zonolive, Santiago, Chile). In addition, silymarin at 500 mg/day PO (Laboratorio Swanson, Valparaíso, Chile) was included to the treatment regimen [16], although the efficacy of silymarin for liver protection has not yet been demonstrated in this species. Throughout the three-months recovery period, the sea lion was evaluated every 15 days by the same veterinarian, who confirmed a positive progression of the condition. Lesions began to remit at the end of the treatment period (day +122), and mycological cure was achieved 15 days later (day+137). Finally, the prescription indicates the permanent use of ozonized water on surfaces, generated by Eko3 equipment (Santiago, Chile).

## 3. Discussion

The genus *Trichophyton* includes around 16 species of both anthropophilic and zoophilic fungi worldwide [13]. Although commonly responsible for superficial mycoses in terrestrial mammals, it has only sporadically been reported as a cause of dermatophytosis in pinnipeds [2]. For instance, *Trichophyton* ssp. Was isolated from the hair and skin of wild northern fur seals *Callorhinus ursinus* hunted in Alaska and California [17]. *T. mentagrophytes*, along with *Malassezia* spp. and *Yarrowia* (*Candida) lipolytica*, was isolated from a group of captive harbor seals and gray seals, where erythematous, thickened, and alopecic skin lesions were primarily found on the face and flippers, particularly around the nail bed [12]. Moreover, *T. rubrum* infection caused multifocal to coalescing, ulcerative, and non-pruritic lesions over the lumbar region in a Patagonian Sea Lion [9]. Persistent “fungal patches” across the body were characteristics of *Trichophyton* dermatitis in Steller sea lions

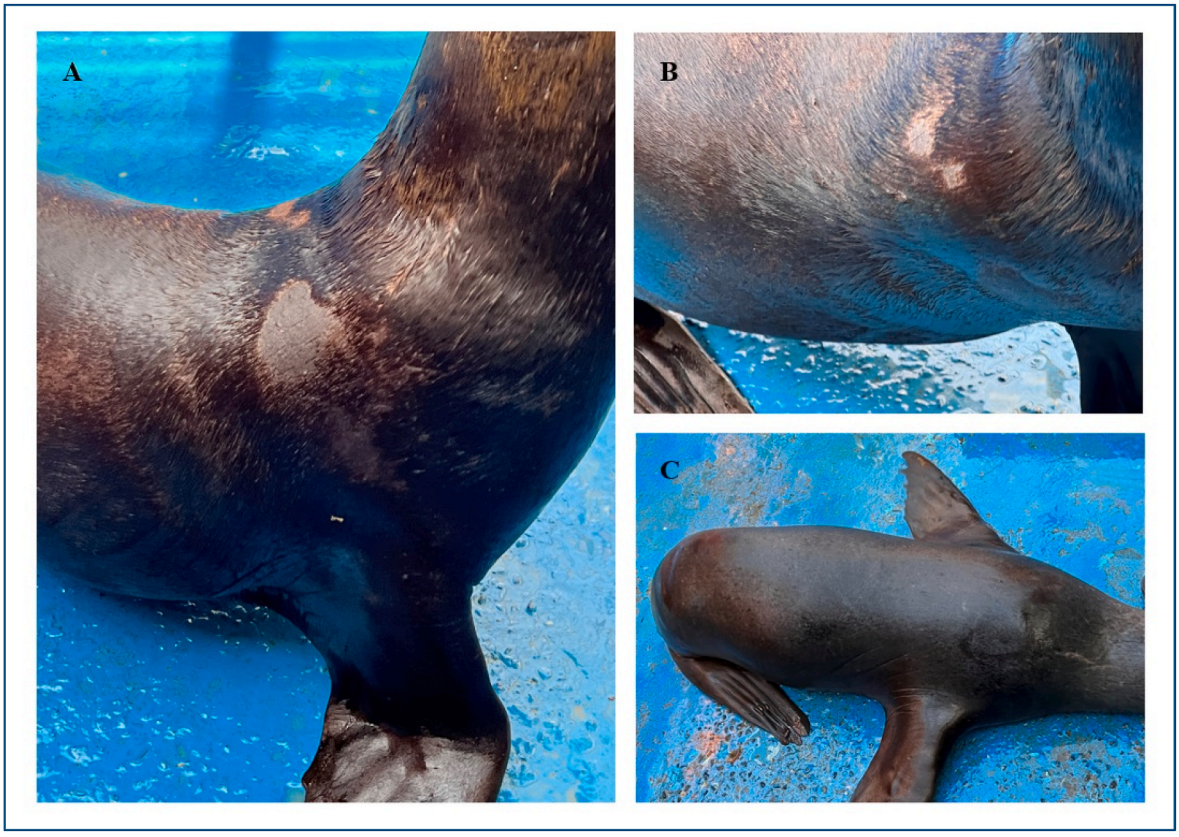


**Fig. 1.** Alopecic lesions presented in a 13-year-old female South American Sea Lion. A: Alopecic lesion located on the back of the animal. B: Smaller alopecic lesions located laterally in the body and C: Appearance at the end of treatment.

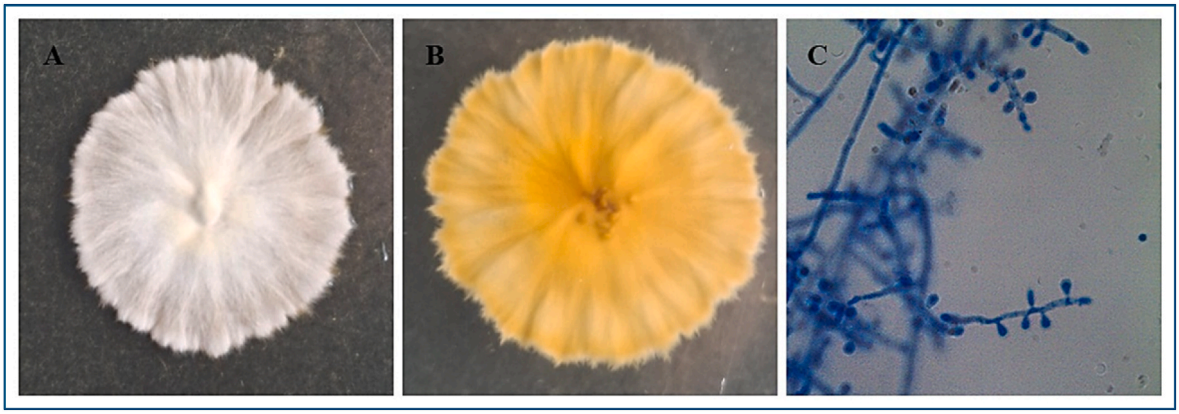


**Fig. 2.** A: *Trichophyton benhamiae* culture on Sabouraud dextrose showing expanding, whitish, flat, and velvety colonies. B: Yellow reverse color. C: Micromorphology showing slender, clavate microconidia at right angle alongside hyphae. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

diagnosed in Alaska [2]. In most cases involving captivity, lesions regression was observed after prolonged treatment with both systemic and topical antifungals [9,11,12].

*Trychophyton benhamiae* is a zoophilic dermatophyte associated with superficial mycoses, mainly described in guinea pigs, although it has also been reported in chinchillas and dogs [18,19]. To date, no previous reports have documented this fungal species affecting pinnipeds. The clinical signs observed were consistent with those previously reported in other mammals. Zoonotic transmission is possible [13] and it can cause highly inflammatory dermatophytosis on glabrous skin and scalp, and less frequently on nails in humans [20,21].

Mechanical disruption of the skin and water quality issues, such as low salinity and/or high temperatures may predispose pinnipeds to fungal infections [2]. These conditions are common in artificial enclosures housing seals and sea lions, particularly in facilities located far from marine water sources where salt must be added to the water. Sometimes, pinnipeds are housed in freshwater pools, which can also contribute to the development of these infections. Another environmental factor that might trigger fungal diseases is the over chlorination of water [12], along with the prolonged use of this chemical for mechanical cleaning and disinfection of pools, despite its many disadvantages [22].

Azoles have been effective in treating *Trichophyton* infections in sea lions [9,12]. However, terbinafine, a drug belonging to the allylamines class, is well-established in the treatment of cutaneous and subcutaneous mycosis [1]. Its broad antifungal spectrum has expanded its use in the therapy of antifungal-resistant strains, particularly in combination with azoles. Due to its hydrophobic properties, terbinafine tends to accumulate in fatty tissues, which is why we recommended the use of a liver restorer such as sylimarin [16] The accurate identification of the causal agent through the isolation and culture of the fungus, along with the genetic identification of the strain and subsequent sensitivity tests, was crucial for the correct selection of the treatment and the successful recovery of the patient.

## CRediT authorship contribution statement

**Ronar López:** Writing – original draft, Methodology, Investigation. **Víctor Silva:** Writing – review & editing, Investigation. **Viviana Bown:** Validation, Methodology, Investigation. **Patricio Godoy-Martínez:** Methodology. **Pamela Thomson:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization.

## Conflict of interest

The authors declare there are no conflict of interest that could influence the work presented.

## Consent

Please declare that you have obtained written and signed consent to publish the case report from the patient or legal guardian(s).

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## Declaration of competing interest

Please declare any financial or personal interests that might be potentially viewed to influence the work presented. Interests could include consultancies, honoraria, patent ownership or other. If there are none state ‘there are none’.

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## References

[1] T.H. Reidarson, D. García-Párraga, N. Wiederhold, Marine mammal mycoses, in: F. M.D. Gulland, L.A. Dierauf, K.L. Whitman (Eds.), CRC Handbook of Marine Mammal Medicine, third ed., CRC Press, Boca Raton, FL, 2018, pp. 389–424.
[2] K.M. Colegrove, K.A. Burek-Huntington, W. Roe, U. Siebert, Pinnipediae, in: Pathology of Wildlife and Zoo Animals, 2018, pp. 570–592, https://doi.org/10.1016/B978-0-12-805306-5.00023-7.
[3] S. Alvarez-Perez, A. Mateos, L. Dominguez, E. Martinez-Nevado, J.L. Blanco, M. E. Garcia, Isolation of Rhodotorula mucilaginosa from skin lesions in a Southern sea lion (Otaria flavescens): a case report, Vet. Med. 55 (2010) 297–301, https://doi.org/10.17221/2988-VETMED.
[4] G. Migaki, S.R. Jones, Mycotic diseases in marine mammals, in: Pathobiology of Marine Mammal Diseases, 2018, p. 27, https://doi.org/10.1201/9781351075411-1.
[5] R.J. Montali, M. Bush, J.D. Strandberg, D.L. Janssen, D.J. Boness, J.C. Whitla, Cyclic dermatitis associated with Fusarium sp infection in pinnipeds, J. Am. Vet. Med. Assoc. 179 (1981) 1198–1202.
[6] L. Reisfeld, P.C. Henrique, B. Schwarz, L. Ippolito, S.D. Coutinho, Alopecia due to Malassezia pachydermatis in a sub-antartic fur seal (Arctocephalus tropicalis), Clinica Veterinaria (2015) 92–96, https://doi.org/10.46958/rcv.2015.XX.n.116.p.92-96.
[7] M.S. Lionakis, D.P. Kontoyiannis, Glucocorticoids and invasive fungal infections, Lancet 362 (2003) 1828–1838, https://doi.org/10.1016/S0140-6736(03)14904-5.

[8] E. Sós, V. Molnár, Z. Lajos, V. Koroknai, J. Gál, Successfully treated dermatomycosis in California sea lions (Zalophus Californianus), J. Zoo Wildl. Med. 44 (2013) 462–465, https://doi.org/10.1638/2011-0275R1.1.
[9] B. Quintard, C. Lohmann, B. Lefaux, A case of Trychophyton rubrum dermatophytosis in a Patagonian Sea Lion (Otaria byronia), J. Zoo Wildl. Med. 46 (2015) 621–623, https://doi.org/10.1638/2014-0214.1.
[10] K. Dukik, G.S. de Hoog, J.B. Stielow, J. Freeke, B.G. van den Ende, V.A. Vicente, S. B.J. Menken, S.A. Ahmed, Molecular and phenotypic characterization of Nannizzia (arthrodermataceae), Mycopathologia 185 (2020) 9–35, https://doi.org/10.1007/s11046-019-00336-9.
[11] P.H. Phillips, P.K. Davenport, D.J. Schultz, Microsporum gypseum dermatophytosis in captive Australian sea lions, Neophoca cinerea, J. Zoo Anim. Med. 17 (1986) 136–138, https://doi.org/10.2307/20094807.
[12] C.G. Pollock, B. Rohrbach, E.C. Ramsay, Fungal dermatitis in captive pinnipeds, J. Zoo Wildl. Med. 31 (2000) 374–378, https://doi.org/10.1638/1042-7260(2000)031[0374:FDICP]2.0.CO;2.
[13] P. Zhan, K. Dukik, D. Li, J. Sun, J.B. Stielow, B. Gerrits van den Ende, B. Brankovics, S.B.J. Menken, H. Mei, W. Bao, G. Lv, W. Liu, G.S. de Hoog, Phylogeny of dermatophytes with genomic character evaluation of clinically distinct Trichophyton rubrum and T. violaceum, Stud. Mycol. 89 (2018) 153–175, https://doi.org/10.1016/j.simyco.2018.02.004.
[14] R.R. Reeves, J.G. Mead, Marine mammals in captivity, in: J.R.J. Twiss, R.R. Reeves (Eds.), Conservation and Management of Marine Mammals, first ed., Smithsonian Institution Press, Washington, D.C., 1999, pp. 412–436.
[15] B. Ahmadi, H. Mirhendi, K. Makimura, G.S. de Hoog, M.R. Shidfar, S. Nouripour-Sisakht, N. Jalalizand, Phylogenetic analysis of dermatophyte species using DNA sequence polymorphism in calmodulin gene, Med. Mycol. 54 (2016) 500–514, https://doi.org/10.1093/mmy/myw004.
[16] M. Trappoliere, A. Caligiuri, M. Schmid, C. Bertolani, P. Failli, F. Vizzutti, E. Novo, C. di Manzano, F. Marra, C. Loguercio, M. Pinzani, Silybin, a component of sylimarin, exerts anti-inflammatory and anti-fibrogenic effects on human hepatic stellate cells, J. Hepatol. 50 (2009) 1102–1111, https://doi.org/10.1016/j.jhep.2009.02.023.
[17] N.A. Vedros, J. Quinlivan, R. Cranford, Bacterial and fungal flora of wild northern fur seals (Callorhinus ursinus), J. Wildl. Dis. 18 (1982) 447–456, https://doi.org/10.7589/0090-3558-18.4.447.
[18] J. Begum, R. Kumar, Prevalence of dermatophytosis in animals and antifungal susceptibility testing of isolated Trichophyton and Microsporum species, Trop. Anim. Health Prod. 53 (2021) 3, https://doi.org/10.1007/s11250-020-02476-3.
[19] A. Peano, V. Hubka, P. Cavana, C. Ottino, M. Blandolino, A.R. Molinar Min, M. Pasquetti, Cases of dermatophytosis caused by Trichophyton benhamiae var. luteum and T. europaeum, newly described dermatophytes within the T. benhamiae complex, Vet. Dermatol. 33 (2022) 440–445, https://doi.org/10.1111/vde.13082.
[20] P. Nenoff, S. Uhrlas, C. Kruger, M. Erhard, U.-C. Hipler, F. Seyfarth, J. Herrmann, T. Wetzig, W. Schroedl, Y. Graser, Trichophyton species of Arthroderma benhamiae - a new infectious agent in dermatology, J Dtsch Dermatol Ges 12 (2014) 571–581, https://doi.org/10.1111/ddg.12390.
[21] J. Tan, X. Liu, Z. Gao, H. Yang, L. Yang, H. Wen, A case of Tinea Faciei caused by Trichophyton benhamiae: first report in China, BMC Infect. Dis. 20 (2020), https://doi.org/10.1186/s12879-020-4897-z.
[22] S. Spotte, Sterilization of Marine Mammal Pool Waters, Animal and Plant Health Inspection Service. US, Department of Agriculture, 1991.