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Yeasts Isolated From *Alouatta palliata*, *Ateles geoffroyi*, *Cebus capucinus* and *Saimiri oerstedii* (Primates: Cebidae)

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Introduction

In the present study the presence of yeasts on the skin, and in the oral cavity and vagina of *Alouatta palliata* (mantled howler monkey), *Ateles geoffroyi* (black-handed spider monkey), *Cebus capucinus* (white-faced capuchin) and *Saimiri oerstedii* Reinhardt, 1872 (red-backed squirrel monkey) from several sites of Costa Rica was examined. These primates have been characterized with regard to their feeding patterns (Jones, 1983; Happel, 1986) and geographical distribution (Massey, 1987; Lippold, 1988, Rodríguez and Chinchilla, 1996). Also, the bacterial flora as well as the endo- and ectoparasites of Costa Rican primates have been reported (Troyo *et al.*, 2002; Calderón-Arguedas *et al.*, 2004; Gamboa-Coronado *et al.*, 2004; Chinchilla *et al.*, 2005; Chinchilla *et al.*, 2006). The present project is unique in being the first to assess the yeast flora in these Neotropical primates. The yeast *Candida* is a saprophyte in natural products, but has been isolated from the mucosa and skin of humans and animals (Mariat and Droulet, 1996). *Candida* is considered an opportunistic micro-organism that causes disease in hosts with a weakened immune system (Ostrosky-Zeichner, 2003). The most common clinical manifestations of candidiasis are cutaneous, mucocutaneous and invasive infections. In humans, *Candida* infections of the mouth and esophagus are frequently associated with AIDS (de Repentigny *et al.*, 2004). Vulvo-vaginal candidiasis is a common cause of vaginal discharge, soreness, vulvar burning, dysuria and local pruritus. *Candida* may be either a commensal or a pathogen of the vagina, which indicates that changes in the host vaginal defense mechanisms or changes in the vaginal micro-environment are generally necessary for *Candida* to induce pathology or association with clinical symptoms (Sobel, 1997). On the other hand, invasive candidiasis is reported in individuals with prolonged neutropenia such as those receiving treatment for leukemia or solid tumors, or transplantation therapy (García-Ruiz *et al.*, 2004).

The finding of yeast in an individual or in a group of healthy monkeys does not imply that this micro-organism is part of the normal flora of the respective species. It is likely, however, that the isolation of *Candida* in a particular population of monkeys is indicative of colonization, as has been well established for other animals (Mariat and Droulet, 1996). The purpose of the present work was thus to

examine the presence of yeasts in the mucosa and skin of Neotropical monkeys.

Methods

Animals and darting procedure

Individuals of *A. palliata*, *C. capucinus*, *A. geoffroyi* and *S. oerstedii* were captured in five areas of the country: Central Pacific (Chomes, Manuel Antonio National Park and Isla San Lucas, Puntarenas), North Pacific (Palo Verde National Park, Guanacaste; San Carlos, Alajuela), Caribean Area (Cahuita, Limón; Sarapiquí, Heredia), South Pacific (Corcovado National Park, Puntarenas) and Central Valley (San Ramón, Alajuela). Captured animals included both adult and juvenile individuals. Detailed capture methods have been described in detail elsewhere (Chinchilla, *et al.*, 2005). Briefly, monkeys were sedated with a dart containing Telazol® (tiletamine and zolazepam), the estimated dose was approximately 20 mg/kg. During the period of restrain, skin, vaginal and oral cavity samples were obtained.

Sampling procedure

Samples were collected between January 2001 and December 2006. Samples from the skin were taken close to the genital area using a scalp and placed into small Petri dishes; samples from the oral cavity and vagina were taken with a sterile cotton swab and placed into a glass test tube containing 10 ml of sterile Sabouraud broth. All samples were then transported to the laboratory for microscopic examination and culture. Samples were centrifuged for 10 min at 5000 RPM and a direct exam with 20% KOH was performed with the pellet for the detection of fungal elements. The pellet was also cultured in Sabouraud glucose agar (SGA) and Mycosel® agar at 22–25ºC for 14 days. Skin samples were directly examined with 20% KOH and cultured as indicated above; positive culture plates were examined with lactophenol cotton blue under the microscope for the detection of fungal elements. Filamentous fungi were identified by micro- and macroscopic morphological characteristics. The Germ tube test was performed on all yeast isolates, and positive samples were identified as *Candida albicans*. Germ tube negative isolates were identified by the commercial API-20X system (Bio Merieux, France).

Results

A total of 608 samples from 275 individuals (157 males, 118 females) were taken for fungal studies; of these samples, 279 were taken from the skin. Direct examination of skin samples did not reveal any fungal elements. Furthermore, cultures of skin samples were negative for fungal pathogens and only common environmental fungi were isolated including zygomycetes, *Penicillium* spp., *Aspergillus* spp., *Hormodendrum* spp., *Fusarium* spp. and *Geotrichum* spp. *Candida* spp. was isolated from 28 out of a total of 228 samples (12.3%) from the oral cavity, and in seven out of a total of 101 samples (6.9%) from the vagina. The relationship between the results of the direct exams and cultures is given in detail in Tables 1 and 2. The yeast isolates, the monkey species from which yeasts were isolated, and the place of collection are given in Table 3 (oral cavity) and Table 4 (vaginal samples). Some cultures of the oral cavity and vagina were positive for environmental fungi, including zygomycetes, *Penicillium* spp., *Aspergillus* spp., *Hormodendrum* spp., *Fusarium* spp., *Sepedonium* spp. *Geotrichum* spp., *Trichosporon* spp. and *Kloeckera* spp.

Discussion

Normal skin may harbor numerous pathogenic fungi. Among the most commonly encountered pathogens of the skin of animals are *Candida* and the dermatophytes, *i. e.*, *Microsporum*, *Trichophyton* and *Epidermophyton* (Zuber and Baddam, 2001). The occurrence of *Trichophyton simii* in primates has been described in India and Brazil (Londero and Benevenga, 1972, Monga and Mohapatra, 1980). In the present study, however, we did not isolate any skin-associated fungi from the samples taken or observed any positive direct exam. Actually, no skin lesions were in the animals studied. On the other hand, in the oral cavity 39.5% of the samples had a positive direct exam. More than half of the fungal cultures were, however, negative. This could be due to overgrowth of common fungal contaminants. *Candida albicans* was the most frequent species isolated in all four species of monkeys studied; *C. albicans* is considered the most frequent yeast isolate of human oral cavity (Teanpaisan and Nittayananta, 1998). *Candida krusei*, *Candida inconspicua*, *Candida lusitaniae* and *Candida prozopfii* followed *C. albicans* in frequency. Interestingly, *C. krusei*, a saprophyte of human oral mucosa, was isolated mostly in a colony of *A. palliata* from Isla San Lucas, Puntarenas, an island occupied as a jail several years ago. In humans, *Candida* species are commensal organisms capable of producing opportunistic infections within the oral cavity only when appropriate predisposing factors exist (Poirier *et al.*, 1997).

Table 1. Relationship between the direct exam and cultures of the oral cavity of 228 monkeys (*A. palliata*, *A. geoffroyi*, *C. capucinus* and *S. oerstedii*).

	Direct exam Positive	Direct exam Positive
Culture positive	28 (12.3%)	
Culture negative	62 (27.2%)	138 (60.5%)

Table 2. Relationship between the direct exam and cultures of vagina of 101 monkeys (*A. palliata*, *A. geoffroyi*, *C. capucinus* and *S. oerstedii*).

	Direct exam Positive	Direct exam Positive
Culture positive	9 (8.9%)	
Culture negative	27 (26.7%)	65 (64.3%)

Table 3. Yeasts isolated from oral cavity, species of monkey and sites of collection.

Yeast (# of isolates)N	Species	Site of collection (# of monkeys)
C. albicans (20)	*A. palliata*	Palo Verde National Park (3)
		Cahuita (5)
		Manuel Antonio National Park (2)
		Sarapiquí (2)
		Isla San Lucas (2)
	C. capucinus	Manuel Antonio National Park (2)
		Sarapiquí (1)
		San Carlos (1)
	S. oerstedii	Manuel Antonio National Park (1)
	A. geoffroyi	San Carlos (1)
C. krusei (4)	*A. palliata*	Isla San Lucas (3)
	C. capucinus	Limón (1)
C. inconspicua (2)	*S. oerstedii*	Corcovado National Park (1)
	A. geoffroyi	Corcovado National Park (1)
C. lusitaniae (1)	*S. oerstedii*	Manuel Antonio National Park
C. prozopfii	*A. palliata*	Sarapiquí

Table 4. Yeasts isolated from vagina, species of monkey and sites of collection.

Yeast (# of isolates)N	Species	Site of collection (# of monkeys)
C. albicans (3)	*A. palliata*	Chomes (2)
		Sarapiquí (1)
C. famata (1)	*C. capucinus*	Sarapiquí
C. inconspicua (1)	*A. geoffroyi*	Corcovado National Park
C. krusei	*A. palliata*	Palo Verde National Park
C. tropicalis (1)	*C. capucinus*	Manuel Antonio National Park

As to the direct exams of the vaginal samples 35.6 % were positive for fungi, but only about 9% were culture positive. Overgrowth of fungal contaminants, as mentioned, may have interfered with the growth of the microscopically observed fungi. As in the oral mucosa, *C. albicans* was the most common yeast isolate in vagina, but it was only isolated from *A. palliata*. The other yeasts isolated from vagina were *C. krusei*, *C. inconspicua*, *C. tropicalis* and *C. famata*. Thus, our findings indicate oral and vaginal *Candida* colonization of a particular monkey or a colony, but cannot establish that these yeast isolates constitute part of the normal oral or vaginal flora in these four species of primates.

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New Sightings of Northern Muriqui (*Brachyteles hypoxanthus*) Females in Forest Fragments Surrounding the Estação Biológica De Caratinga-RPPN Feliciano Miguel Abdala, Minas Gerais, Brasil

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The northern muriqui (*Brachyteles hypoxanthus*) is a critically endangered primate with about 1,000 individuals distributed among 12 remaining populations (Mendes *et al.*, 2005). Nearly 300 individuals, representing nearly a third of the entire species, are distributed in the four mixed-sex groups at the RPPN Feliciano Miguel Abdala (RPPN-FMA; previously known as the Estação Biológica de Caratinga), a privately protected forest fragment of roughly 1,000 ha (updated from Strier *et al.,* 2006). This population has been monitored systematically since 1982, when it was estimated to consist of just 40–50 individuals (Valle *et al.*, 1984). Hunting had long been prohibited at this site, and the six-fold increase documented in the size of the population in less than 30 years can be attributed, at least in part, to improved habitat protection. There has been some habitat recovery within and around the RPPN-FMA due to the regeneration of small parcels of land that had previously been cleared for small coffee plantations and pasture, but the growth of the muriqui population has far exceeded the expansion of the forest.

Previous analysis of this population's potential long-term viability identified the need for increasing the amount of suitable habitat available to this growing population (Strier, 1993/1994). The establishment of ecological corridors to connect neighboring forest fragments with the protected forest in the RPPN-FMA has been a critical component of ongoing and long-term management plans for this species (Strier and Fonseca, 1996/1997; Rylands *et al.*, 1998). However, until recently, nothing was known about the accessibility of these surrounding forest fragments or whether they could support muriquis. Here we report the first confirmed sightings of four female northern muriquis (1 adult and 3 subadults) in three of the fragments. The new findings indicate that these fragments provide a minimum structure for supporting muriquis and represent key areas for the establishment of the corridor.

Systematic censuses were conducted in eight forest fragments surrounding the RPPN-FMA between June 2008-October 2009; muriquis were sighted in three of these fragments (Figure 1). On 30 June 2008 a solitary adult female was encountered in one fragment (19° 45' 54" S, 41° 49' 23" W). The next year, on 23 July 2009,