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ARTICLE

Yeast microbiota of natural cavities of manatees (*Trichechus inunguis* and *Trichechus manatus*) in Brazil and its relevance for animal health and management in captivity

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Abstract: The aim of this study was to characterize the yeast microbiota of natural cavities of manatees kept in captivity in Brazil. Sterile swabs from the oral cavity, nostrils, genital opening, and rectum of 50 *Trichechus inunguis* and 26 *Trichechus manatus* were collected. The samples were plated on Sabouraud agar with chloramphenicol and incubated at 25 °C for 5 days. The yeasts isolated were phenotypically identified by biochemical and micromorphological tests. Overall, 141 strains were isolated, of which 112 were from *T. inunguis* (*Candida albicans*, *Candida parapsilosis* sensu stricto, *Candida orthopsilosis*, *Candida metapsilosis*, *Candida guilliermondii*, *Candida pelliculosa*, *Candida tropicalis*, *Candida glabrata*, *Candida famata*, *Candida krusei*, *Candida norvegensis*, *Candida ciferri*, *Trichosporon* sp., *Rhodotorula* sp., *Cryptococcus laurentii*) and 29 were from *T. manatus* (*C. albicans*, *C. tropicalis*, *C. famata*, *C. guilliermondii*, *C. krusei*, *Rhodotorula* sp., *Rhodotorula mucilaginosa*, *Rhodotorula minuta*, *Trichosporon* sp.). This was the first systematic study to investigate the importance of yeasts as components of the microbiota of sirenians, demonstrating the presence of potentially pathogenic species, which highlights the importance of maintaining adequate artificial conditions for the health of captive manatees.

Key words: colonization, sirenians, *Candida* spp., *Trichosporon* sp., *Rhodotorula* sp.

Résumé : L'objectif de la présente étude était de caractériser la microflore de levures de cavités naturelles de lamantins tenus en captivité au Brésil. On a effectué des prélèvements par écouvillonnage de la cavité orale, des narines, de l'orifice génital et du rectum de 50 *Trichechus inunguis* et de 26 *Trichechus manatus*. On a ensemencé les échantillons sur des géloses Sabouraud avec chloramphénicol pour ensuite les incuber à 25 °C pendant 5 jours. Les levures ainsi isolées ont été identifiées phénotypiquement au moyen de tests biochimiques et micromorphologiques. Dans l'ensemble, 141 souches ont été isolées, dont 112 issues de *T. inunguis* (*Candida albicans*, *Candida parapsilosis* sensu stricto, *Candida orthopsilosis*, *Candida metapsilosis*, *Candida guilliermondii*, *Candida pelliculosa*, *Candida tropicalis*, *Candida glabrata*, *Candida famata*, *Candida krusei*, *Candida norvegensis*, *Candida ciferri*, *Trichosporon* sp., *Rhodotorula* sp., *Cryptococcus laurentii*) et 29 de *T. manatus* (*C. albicans*, *C. tropicalis*, *C. famata*, *C. guilliermondii*, *C. krusei*, *Rhodotorula* sp., *Rhodotorula mucilaginosa*, *Rhodotorula minuta*, *Trichosporon* sp.). Il s'agit de la première étude systématique examinant

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l'importance des levures à titre d'éléments de la microflore de siréniens, laquelle démontre du coup la présence d'espèces potentiellement pathogènes et met en évidence l'importance de maintenir des conditions artificielles propices à la bonne santé des lamantins en captivité. [Traduit par la Rédaction]

Mots-clés : colonisation, siréniens, *Candida* spp., *Trichosporon* sp., *Rhodotorula* sp.

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Introduction

The Order Sirenia consists of 4 living species of herbivorous aquatic mammals, the manatees and dugongs, all of which are classified as vulnerable to extinction (Marsh et al. 2011; IUCN 2013). In Brazil, there are 2 species, the Amazonian manatee (*Trichechus inunguis*) and the West Indian manatee (*Trichechus manatus*), both threatened by hunting, bycatch, habitat destruction, collisions with vessels, and other impacts of anthropogenic origin (ICMBio 2011).

In addition to the high mortality rates due to human activities, sirenians are exposed to infectious agents that cause natural mortality (Vergara-Parente et al. 2003*a*; Bossart et al. 2004; Bonde et al. 2004). Bacteria are the main agents diagnosed in manatees and dugongs and these microorganisms can cause localized or systemic and fatal diseases (Forrester 1992; Sato et al. 2003; Vergara-Parente et al. 2003*a*; Bossart et al. 2004; Nielsen et al. 2013). In relation to viral diseases, the main one is cutaneous papillomatosis, causing papillomavirus in *T. manatus* (Woodruff et al. 2005). Fungi are rarely diagnosed, although they have been isolated from cases of dermatitis, dermatophytosis, and phaeohyphomycosis (Dilbone 1965; Forrester 1992; Sidrim et al. 2015).

Little is known about the impact of infectious diseases on the sirenian population. Even though manatees are remarkably resilient to natural diseases, as a result of an efficient and responsive immune system, alterations in the aquatic environment caused by climate change, habitat destruction, and pollution can make these coastal animals more susceptible to infectious agents (Bonde et al. 2004; Bossart 2011; Sulzner et al. 2012).

It is important to highlight the role of microorganisms as members of the microbiota, assisting in the development of the immune response and forming barriers in the skin and mucous membranes. In addition, the microbiota is essential for the utilization of nutrients in the digestive tract, especially in herbivores (Walter et al. 2011; Hooper et al. 2012; Sanford and Gallo 2013). Despite this importance, little is known about the microbiota of sirenians. Some papers have been published discussing the bacterial microbiota of West Indian manatees and dugongs, but these papers are without data on the yeast microbiota of these animals (Vergara-Parente et al. 2003*b*; Eigeland et al. 2012; Merson et al. 2013; Attademo 2014).

In this context, the aim of this study was to compare the composition of the yeast microbiota of the respiratory, digestive, and genital tracts of the 2 living species of sirenians in Brazil, the Amazonian manatee and the West Indian manatee, from animals held in captivity for rehabilitation and release.

Materials and methods

Ethical aspects

This study was authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), through the license SISBIO No. 35950-1, and by the Animal Research Ethics Committee of Universidade Estadual do Ceará, under the No. 12639479-2.

Collection of samples

Sterile swabs were inserted into the natural cavities (oral cavity, nostrils, genital opening, and rectum) of 50 Amazonian manatees (*T. inunguis*) and 26 West Indian manatees (*T. manatus*) held in captivity in 3 Brazilian institutions that have rescue, rehabilitation, and release programs with these species: Centro de Preservação e Pesquisa de Mamíferos Aquáticos (CPPMA)/Eletrobras Amazonas Energia, in the State of Amazonas; Centro Mamíferos Aquáticos (CMA/ICMBio), in the State of Pernambuco; and Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (AQUASIS), in the State of Ceará.

The manatees belonged to both sexes and different age groups (newborns, calves, juveniles, and adults) (Table 1). The animals received different diets (artificial milk formulas or vegetables), according to age and internal institutional protocols. All animals received a single clinical score (poor, medium, good, or excellent) considering the nutritional status, presence of external lesions, clinical history, and use of antimicrobials. Conditions for maintenance in captivity also varied, such as water treatment (chlorinated or untreated), salinity (salt or fresh), and density of animals per tank.

Microbiological evaluation

For isolation of yeasts, we collected 163 sterile swabs from the oral cavity (n = 50), nostrils (n = 50), genital opening (n = 32), and rectum (n = 31) of *T. inunguis* and 75 sterile swabs from the oral cavity (n = 26), nostrils (n = 26), genital opening (n = 12), and rectum (n = 11) of *T. manatus*. The swabs were plated in Petri dishes containing Sabouraud dextrose 2% agar plus chloramphenicol (0.5 g/L) and incubated at 25 °C, for a period of 5 days. Microbial colonies that resembled yeast colonies were stained with cotton blue-lactophenol and observed under an optical microscope (40×) to verify the presence of blastoconidia, hyphae, or pseudohyphae (De Hoog et al. 2000; Brilhante et al. 2010, 2011).

The identification of *Candida* spp. was based on phenotypical characteristics, such as macromorphological and

Table 1. Characterization by species, sex, and age class of the specimens evaluated.

		No. of individuals by:					
		Sex		Age class			
Species	*n*	M	F	Newborn	Calf	Juvenile	Adult
Trichechus inunguis	50	24	26	0	6	15	29
Trichechus manatus	26	11	15	5	2	11	8
Total	76	35	41	5	8	26	37

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micromorphological analyses on cornmeal + Tween 80 agar. Additionally, biochemical tests were performed, such as urease production, carbohydrates and nitrogen assimilation, and carbohydrate fermentation. All *Candida* isolates were plated on chromogenic medium CHROM-agar Candida (Difco, USA) for identification of mixed colonies. The identification of other yeast species was performed according to specific protocols. Isolates of *Trichosporon* sp. were identified on the basis of macromorphology and micromorphology on 2% malt extract agar and biochemical tests, such as the production of urease and carbohydrate and nitrogen assimilation. Colonies of *Cryptococcus* spp. and *Rhodotorula* spp. were evaluated by macromorphology and micromorphology on cornmeal + Tween 80 agar and 2% malt extract agar, respectively, by biochemical profile (urease production and auxonogram), and through the automated system Vitek 2 (De Hoog et al. 2000; Brilhante et al. 2010; Kurtzman et al. 2011).

It is important to highlight that the identification of *C. albicans* was confirmed through the methodology described by Ahmad et al. (2012), as part of a parallel research of our group. Isolates belonging to *C. parapsilosis* complex were molecularly identified on the basis of the amplification of a partial sequence of the secondary alcohol dehydrogenase gene, as described by Tavanti et al. (2005) and Brilhante et al. (2014). Finally, 6 representatives of each *Trichosporon* sp. phenotype were submitted to sequence analysis of the regions IGS1 and ITS1 and the domains D1 and D2 (Fell et al. 2000; Rodriguez-Tudela et al. 2005).

Statistical analysis

The positivity rate between the 2 species of manatee and the recovery of different yeast genera from each evaluated manatee species was compared through Fisher's exact test, using a significance level of 5%.

Results

Yeast isolation

Trichechus inunguis

We obtained 112 yeast samples from 4 anatomical sites of *T. inunguis*, with 82% (41/50) of the animals being positive. The isolation rates were 68% (34/50) for the oral cavity, 10% (5/50) for the nostrils, 84.3% (27/32) for the genital opening, and 67.7% (21/31) for the rectum.

Four genera of yeasts were identified in samples from *T. inunguis* (Table 2): *Candida* represented 72.3% (81/112) of the isolates, followed by *Trichosporon* with 25% (28/112), *Rhodotorula* with 1.78% (2/112), and *Cryptococcus* with 0.89% (1/112). Twelve species of *Candida* were recovered, with *Candida albicans* as the most commonly isolated species of yeast (40/112). The non-albicans *Candida* species obtained were *C. parapsilosis* sensu stricto (14/112), *C. orthopsilosis* (4/112), *C. metapsilosis* (3/112), *C. guilliermondii* (9/112), *C. pelliculosa* (3/112), *C. tropicalis* (2/112), *C. glabrata* (2/112), *C. famata* (1/112), *C. krusei* (1/112), *C. norvegensis* (1/112), and *C. ciferri* (1/112). One isolate of *Cryptococcus laurentii* and 2 of *Rhodotorula* sp. were obtained.

Mixed colonization in the same anatomical site was noted in 20 positive samples obtained from 16 *T. inunguis*. Two species of yeasts were isolated from 6 oral samples, 1 nostril sample, 4 genital openings samples, and 4 rectal samples. Up to 3 different yeast species were isolated from 5 rectal swabs. Among these animals, 3 individuals had mixed colonization in 2 anatomical sites (Table 3).

Trichechus manatus

We obtained 29 yeasts from the 4 anatomical sites of *T. manatus*. Out of the 26 tested animals, 20 (76.9%) were positive for yeast recovery. The isolation rates were 42.3% (11/26) for the oral cavity, 19.2% (5/26) for the nostrils, 50% (6/12) for the genitourinary tract, and 45.4% (5/11) for the rectum. Overall, the recovery rate for yeasts from *T. manatus*, considering all tested anatomical sites, was statistically lower than that obtained for *T. inunguis* ($P < 0.05$).

In *T. manatus* we identified 3 genera of yeasts (Table 2): *Candida* represented 82.7% (24/29) of isolates, followed by *Rhodotorula* with 13.8% (4/29), and *Trichosporon* with 6.89% (1/29). The recovery rates of *Rhodotorula* sp. and *Trichosporon* sp. from *T. manatus* were, respectively, higher and lower than those obtained for *T. inunguis* ($P < 0.05$). The species identified were *C. albicans* (12/29), *C. tropicalis* (4/29), *C. famata* (4/29), *C. guilliermondii* (3/29), *C. krusei* (1/29), *Rhodotorula mucilaginosa* (2/29), and *Rhodotorula minuta* (1/29).

Mixed colonization was observed in only 1 sample from *T. manatus*, in which 3 different *Candida* species were isolated from the oral cavity of the same individual (Table 3).

Physiological and environmental factors

In relation to the clinical score of *T. inunguis*, 88% (44/50) of animals received an excellent score, 8% (4/50) a good score, and 4% (2/50) a medium score. CFU counts of >50 were obtained only from animals with excellent and good scores. Considering the age factor, at least 1 yeast species was isolated from 100% (6/6) of calves and juveniles (15/15) and from 69% (20/29) of adults. Regarding gender, the recovery rate from males (95.8%; 23/24)

Table 2. Yeasts species isolated from *Trichechus inunguis* and *Trichechus manatus* according to the anatomical site.

	No. (%) isolated from:									
	Trichechus inunguis					*Trichechus manatus*				
Yeast species	Oral cavity	Nostrils	Genitals	Rectum	Total	Oral cavity	Nostrils	Genitals	Rectum	Total
Candida albicans	26 (65)	—	11 (35.6)	3 (8.6)	40 (35.7)	9 (69.2)	3 (60)	—	—	12 (41.8)
Candida parapsilosis sensu stricto	3 (7.5)	2 (33.2)	4 (13)	5 (14.4)	14 (12.5)	—	—	—	—	—
Candida orthopsilosis	1 (2.5)	—	1 (3.2)	2 (5.7)	4 (3.7)	—	—	—	—	—
Candida metapsilosis	—	1 (16.7)	—	2 (5.7)	3 (2.6)	—	—	—	—	—
Candida guilliermondii	2 (5)	—	3 (9.6)	4 (11.5)	9 (8)	1 (7.7)	—	1 (16.7)	1 (20)	3 (10.3)
Candida pelliculosa	—	—	1 (3.2)	2 (5.7)	3 (2.6)	—	—	—	—	—
Candida tropicalis	—	—	1 (3.2)	1 (2.8)	2 (1.8)	1 (7.7)	1 (20)	2 (33.3)	—	4 (13.7)
Candida glabrata	—	1 (16.7)	—	1 (2.8)	2 (1.8)	—	—	—	—	—
Candida famata	—	—	—	1 (2.8)	1 (0.9)	2 (15.4)	—	—	2 (40)	4 (13.7)
Candida ciferri	—	1 (16.7)	—	—	1 (0.9)	—	—	—	—	—
Candida norvegensis	—	—	1 (3.2)	—	1 (0.9)	—	—	—	—	—
Candida krusei	—	—	1 (3.2)	—	1 (0.9)	—	—	—	1 (20)	1 (3.4)
Trichosporon sp.	8 (20)	1 (16.7)	7 (22.6)	12 (34.3)	28 (25)	—	1 (20)	—	—	1 (3.4)
Rhodotorula sp.	—	—	—	2 (5.7)	2 (1.8)	—	—	—	1 (20)	1 (3.4)
Rhodotorula mucilaginosa	—	—	—	—	—	—	—	2 (33.3)	—	2 (6.9)
Rhodotorula minuta	—	—	—	—	—	—	—	1 (16.7)	—	1 (3.4)
Cryptococcus laurentii	—	—	1 (3.2)	—	1 (0.9)	—	—	—	—	—
Total	40	6	31	35	112	13	5	6	5	29

Table 3. Mixed colonization according to the anatomical site.

	No. of samples in:				
	Trichechus inunguis		*Trichechus manatus*		
Anatomical site	2 species	3 species	2 species	3 species	Total
Oral cavity	6	0	0	1	7
Nostrils	1	0	0	0	1
Genitals	4	0	0	0	4
Rectum	5	5	0	0	10

was statistically higher than that observed for females (69.2%; 18/26) ($P < 0.05$).

In the *T. manatus* assessment, 69.3% (18/26) received an excellent clinical score, 11.5% (3/26) a good score, 11.5% (3/26) a medium score, and 7.7% (2/26) a poor score. As in *T. inunguis*, CFU counts of >50 were obtained only in animals with excellent and good scores. Considering the age factor, at least 1 yeast species was isolated from 60% (3/5) of newborns, 100% (2/2) of calves, 72.7% (8/11) of juveniles, and 87.5% (7/8) of adults. Regarding gender, the recovery rate from males (90.9%; 10/11) was statistically higher than that observed for females (66.6%; 10/15) ($P < 0.05$).

Considering that most species of yeasts (*C. guilliermondii*, *C. parapsilosis stricto sensu*, *C. famata*, *C. albicans*, and *C. tropicalis*) isolated from sirenians with medium or poor clinical score were also present in healthy animals, they were considered as components of the microbiota. Only 1 isolate of *C. krusei* (1/112) was obtained from the genital opening of a *T. inunguis* calf, which had been subjected to a surgical procedure in the genital area 1 day before the collection. Concerning *T. manatus*, 66.6% (2/3) of the animals from which *Rhodotorula* spp. were recovered were immunocompromised. The healthy specimen that was also positive for this genus was in a tank beside the animal with *Rhodotorula* sp. in 2 anatomical sites. Thus, it is suggested that *C. krusei* and *Rhodotorula* sp. are not normal components of the microbiota of *T. inunguis* and *T. manatus*, respectively.

The isolation rates tended to be higher in tanks with a density of more than 10 manatees (52.1%), when compared with the isolation rates of tanks containing from 2 to 5 individuals (42.2%) and with only 1 individual (38.4%). In the majority of the tanks with more than 3 animals, they shared 1 predominant species or genus of yeast in the same anatomical sites (Table 4).

Discussion

Among the few publications on the characterization of the microbiota of sirenians, this is the first systematic evaluation for the isolation of yeasts in such animals. Previously, only the study of Nielsen et al. (2013) showed the isolation of yeasts from sirenians. They obtained 2 strains of *Candida* spp., one from the skin and the other from feces, and 1 strain of *Cryptococcus humicola* from the nostrils of dugong carcasses in Australia.

In the present study, the mucous membranes of natural cavities of *T. inunguis* and *T. manatus* were colonized by yeasts of different species, demonstrating that these microorganisms are part of the microbiota of the oral cav-

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Table 4. Predominant species of yeast in the anatomical sites of animals in the same tank.

Tank	Host	No. of animals	Predominant yeast species found in: Oral cavity	Nostrils	Genitals	Rectum
A	*T. inunguis*	16	*C. albicans* 76.9% (10/13)	*C. albicans* 66.6% (2/3)	*C. albicans* 61.5% (8/13)	*C. parapsilosis* sensu stricto 50% (5/10)
B	*T. inunguis*	16	*C. albicans* 100% (9/9)		*Trichosporon* sp. 44.4% (4/9)	*Trichosporon* sp. 77.7% (7/9)
C	*T. inunguis*	12	*C. albicans* 66.6% (4/6)			
D	*T. manatus*	5	*C. albicans* 100% (3/3)			
E	*T. manatus*	4	*C. albicans* 100% (4/4)			

Note: Data are the species name, followed by the percentage, as calculated by the number of positive animals for a given yeast species / number of positive animals for a given anatomical site (in parentheses).

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ity, nostrils, genital opening, and rectum. Considering that our goal was to characterize the yeast microbiota of the natural cavities of manatees and that *Malassezia* species are mainly part of the microbiota of skin surfaces rather than mucosal surfaces (Cabañes 2014), the recovery of nonlipophilic yeast species was prioritized. Thus, the clinical specimens were seeded on Sabouraud agar, which possibly restricted the growth of several *Malassezia* spp.

The yeast microbiota of *T. inunguis* was quantitatively more representative and diverse than that of *T. manatus*. Differences in salinity and chemical treatment of water may be associated with this finding, since the Amazonian manatees were kept in chlorine-free fresh water, while the West Indian manatees were kept in tanks with chlorinated saltwater. Most of the evaluated animals were rescued and taken to rehabilitation when they were newborns or calves, decreasing the chances of colonization by yeasts during the brief stay in the natural environment and during the brief contact with their mothers. At the 3 surveyed institutions, there was human contact with animals during biomedical management and feeding, especially with lactating calves. Other possible factors that may explain the higher rates of isolation in *T. inunguis* are the proximity of the tanks to the surrounding flora and other wild animals, particularly birds, which are kept in rehabilitation at CPPMA. According to Martins et al. (2002) and Buck et al. (2006), artificial environmental conditions; the use of antibiotics; inadequate water composition; and contact with terrestrial animals, humans, and the surrounding flora are factors that favor greater prevalence of yeasts in captive marine mammals.

Candida was the most isolated yeast genus from both host species, with emphasis on *C. albicans*, which was the most prevalent yeast species in both Amazonian and West Indian manatees. In other aquatic mammals, like bottlenose dolphins, *Candida* spp. are more prevalent in captive animals than wild ones, with *C. albicans* being the main species isolated (Buck 1980; Buck et al. 2006; Avalos-Téllez et al. 2010).

Yeasts of the genus *Trichosporon* were isolated from all anatomical sites of the Amazonian manatees, indicating these animals have greater potential for colonization. A pilot study conducted by us on the molecular identification of these isolates revealed a high prevalence of *Trichosporon asahii*. The lower tolerance of most species of *Trichosporon* to saline environments, with the exception of *T. mucoides* (Gunde-Cimerman et al. 2009), may have contributed to the low prevalence in West Indian manatees, which are kept in saltwater pools. The opposite was observed with respect to the genus *Rhodotorula*, which includes several halotolerant species (Butinar et al. 2005); hence, this genus was more prevalent in the West Indian manatees than in the Amazonian manatees.

The isolation rates were higher in tanks with more than 10 animals, and frequently, these animals shared at least one predominant species of yeast in their anatomical sites. Mixed colonization in an anatomical site of the same individual was observed primarily in the Amazonian manatees and in tanks with a high density of animals. This finding might indicate increased contamination of water in such enclosures due to the high animal density, so we recommend an improved water treatment system in these rehabilitation centers to minimize the risks of opportunistic yeast infections.

Among the surveyed anatomical sites, the highest prevalence of yeasts was observed in the genital opening of both host species. Fewer genital and rectal swabs were collected owing to operational difficulties in restraining larger animals, mostly females and the West Indian manatees, which may have led to underestimating the yeast isolation rates in these anatomical sites and this gender. The oral cavity was more colonized by *C. albicans* in both hosts. In the genital and rectal opening of *T. inunguis*, there was greater diversity of species of yeasts, especially *Candida* spp. The genital mucosa contained a higher prevalence of *C. albicans*, *Trichosporon* sp., and *C. parapsilosis* sensu stricto, while in the rectal mucosa, *Trichosporon* sp. was the most prevalent genus. In these same anatomical sites of *T. manatus*, few isolates were obtained owing to limitations in the collection procedure, which complicates the determination of the most important yeast species in the microbiota. *Rhodotorula* sp. and *C. tropicalis* were more prevalent in the genital opening and *C. famata* was more frequently found in the rectum. Few yeast species were isolated from the nostrils of both hosts, possibly owing to less contact of the nasal mucosa with water. Manatees remain submerged most of the time, with their nostrils closed (Marsh et al. 2011).

Overall, there was no influence of the clinical score on the isolation of yeasts and CFU counts in the 2 host spe-

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cies, unlike what was observed by Brilhante et al. (2010) in a similar study with cockatiels, which revealed higher CFU counts in animals with low clinical scores. However, the only neonates colonized (both *T. manatus*) had low clinical scores. The higher predisposition in these individuals is possibly due to the immunosuppression in animals at this age group and early contact with artificial conditions. Thus, we believe that isolation of yeasts from neonates could be a potential indicator of poor health. The other age groups were similarly susceptible to colonization. With respect to gender, males of both species had higher rates than females. However, these values may not reflect a real difference between genders, since among the animals from which genital and rectal swabs were not collected, 81.25% (26/32) were females.

Although yeasts are commensal microorganisms belonging to the normal microbiota, they are capable of causing severe diseases in humans and animals, particularly when there is an impairment of the immune function (Brilhante et al. 2010). The occurrence of occupational zoonoses caused by yeasts has not been reported in institutions that deal with aquatic mammals. However, during this study, there were 4 cases of onychomycosis caused by yeasts (*C. albicans*, *C. famata*, and *T. asahii*) in people involved in the management of Amazonian manatees in captivity. All species involved were also isolated from the animals. Takahashi et al. (2010) mentioned the risk of transmission of *Candida* spp. for people with close contact with dolphins in captivity. Occupational zoonoses due to the interaction between marine mammals and humans may increase the exchange of pathogens between these hosts and contribute to the emergence of infectious diseases (Hunt et al. 2008).

Despite the extensive knowledge on the biology of some species of sirenians, there is still little information on the health aspects in many areas of occurrence (Bonde et al. 2004; Marsh et al. 2011). In Brazil, studies on the health of *T. inunguis* and *T. manatus* in wild and captivity are listed as priorities in the National Action Plan for Conservation of Sirenians, since some subpopulations are more endangered regionally than globally (ICMBio 2011). The isolation of yeast microbiota increases the range of possible etiologic agents of infectious diseases in these animals and demonstrates the strong influence of artificial conditions on the health of manatees. Given that most of the animals, especially *T. manatus*, will be released into the wild, we recommend including monitoring of fungal and bacterial microbiota of captive and free-ranging manatees as a tool to better understand their role in the health of wild populations.

Conclusions

Natural cavities of *T. inunguis* and *T. manatus* in captivity are colonized by yeasts of different species, most likely acquired in the artificial environment. Some of the isolated species are important opportunistic pathogens that can cause serious and fatal diseases, mainly in immunosuppressed individuals.

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