**Research note**

Running title: Cloacal mycobiota in broud-snouted caiman

Cloacal mycobiota in wild females of *Caiman latirostris* (Crocodylia: Alligatoridae)

Micobita Cloacal de hembras de *Caiman latirostris* (Crocodylia: Alligatoridae) en estado silvestre.

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**Abstract.** There are a few reports about the existence of cloacae mycobiota on wild reptilesand fungal presence and function in*Caiman latirostris*remains unknown. Our objective was to describe fungal community present in the cloaca of wild females’ of broad-snouted caiman during its reproductive season and its relationship with female’s body condition.Fungi were found in 9 out of 13 cloacal samples and 14 species of fungi were isolated and identified. Three taxa of the total species listed had a higher occurrence values, two of them are pathogenic fungi.In this case Body Condition Index has no relationship with fungi frequency; the permanent contact of *C. latirostris* females’ cloaca with the habitat soil and nest substrate could be a source for fungi found in this study.The findings in this work support the theory of reptiles as facultative carriers for fungi.

Keywords: Broad-snouted caiman, filamentous fungi, wild reptiles, Yeasts, pathogens.

**Resumen.** Hay pocos reportes acerca de la micobiota cloacal en reptiles silvestres y en particular, la presencia de hongos y su función en *Caiman latirostris* es aún desconocida. El objetivo de este trabajo fue describir la comunidad fúngica presente en la cloaca de hembras silvestres de yacaré overo durante su época reproductiva y analizar si la cantidad de hongos tiene alguna relación con la condición corporal de la hembra. Las hembras que resultaron positivas para hongos fueron 9 de las 13 muestreadas y fueron 14 las especies de hongos aisladas e identificadas. Del total de especies listadas, tres tienen los valores de abundancia más altos y dos de ellas son reconocidas como hongos patógenos. La condición corporal no tuvo relación con la frecuencia de hongos en general. Los hongos identificados pudieron tener su origen en el contacto permanente de la cloaca de la hembra de *C. latirostris* con el suelo del hábitat y/o el sustrato del nido. Los resultados de este trabajo apoyan la teoría de que los reptiles son transportadores facultativos de hongos y/o sus esporas.

Palabras clave: Yacaré Overo, hongos filamentosos, reptiles silvestres, levaduras, patógenos.

Fungi are considered ubiquitous organisms in nature; therefore, it is common to isolate fungi from tissues (e.g., skin, intestines and lungs) that are in contact with water and soil in different reptiles species(Mitchell and Tully, 2009). Although some information available for marine turtles (Phillot et al., 2002) and giant lizards (genus *Gallotia*; Martinez Silvestre et al., 2003) related to the cutaneous or intestinal mycobiota of reptiles are poorly known.Some authors suggest that reptiles’ mycobiota(both cutaneous and gastrointestinal), could be rich and varied (Austwickand Keymer, 1981;Migakiet al., 1984).Information describing mycobiota in wild crocodilians remains unknown (Mitchell and Tully, 2009). Under captive conditions, some cutaneous and gastrointestinal fungi have been isolated from crocodilians (Thomas et al., 2002; Mitchell and Tully, 2009); these mycobiota were associated with disease, whereas others were incidental findings (Jacobsonet al., 2000).

Studies describing and identifying mycobiotain wild animals are the first step to understand how they could affect the survival of wild populations (Jacobson, 2007). The proportions of mycotic diseases in animals that are regularly communicable or contagious are small(Jacobson et al., 2000). Most fungi are opportunistic invaders of the integument, respiratory system, and gastrointestinal tract and may become pathogenic with changes in the immnunological or ecological status of the animal host (Jacobson, 2007). Previously, some studies showed a negative relationship between the body condition and fungi presence in reptiles (Buenviaje et al.,1998; Miller et al., 2004; Gartrell and Hare, 2005). In this sense it should be expected that in caimans a poor body condition would be related to the presence of higher abundance of fungi.

Mycotic diseases are generally acquired from non-animal sources in the environment; and most mycotic disease agents have other, usually saprobic, ecological roles through which they exert their main ecological impact (Ghirardi et al., 2011).In this context the aim of our study was to describe thefungifoundinthe cloacaof broad-snouted caiman (*C.latirostris*)females during their reproductive stage and if a poor body condition is linked with higher fungi frequencies on the females.

Samples were obtained during the reproductive season of broad-snout caimanfrom January to February 2011 in Santa Fe Province, Argentina, in the following sites: Fisco (n = 8; 30°11'43.56"S, 61°0'38.94"W); Cacique (n = 2; 30°38'1.00"S, 60°17'34.50"W); Los Saladillos (n = 2; 30°42'59.5''S, 60°17'47.3''W) and Espín (n = 1; 29°56'12.94"S, 60° 3'43.85"W). Females (mean Total Length: 155.7 ± 9.2 cm, SVL: 79.3 ± 2.9 cm and Body Mass: 16.5 ± 1.9 gr)were captured next to their nests at their natural habitats (n = 13).

Theswabsampleswere takenin theedgeinside of thecloaca(ca2cmdeep)ofeachanimal by connectingone endofswab to the linning of the cavity where the clitoris is laying (NuñezOtaño et al., 2010).Striations were performed with the swabs in petri dishes with culture medium, the agar contained streptomycin (5 mg/ml) and chloramphenicol (2.5 mg/ml) to avoid excessive bacterial growth. The plates were maintained under natural conditions of temperature and photoperiod and were examined macroscopically (Wild Heerbrugg – Plan 1x) and microscopically (Leitz-Dialux 20 EB) after a minimum of seven days. Identiﬁcation was achieved by taxonomic processes such as direct comparison of specimens and by the use of taxonomic keys, descriptions and illustrations. As an individual we consider one CFU (colony forming unit) of fungi. Cultures were labeled as LPSC 1103 through LPSC 1120 in the culture collection of the Institute Carlos Spegazzini (La Plata, Buenos Aires, Argentina).Nutritional modes have been used as a means of delimitating econutritional categories of behavior according to whether fungi were biotrophic-pathogen or biotrophic-saprotrophs (Cooke and Whipps, 1993). Body condition index (BCI) was considered as the residuals of the linear regression between bodies size (SVL in our case) and body mass, as commonly used (Litzguset al., 2008). Analyses for SVL and body mass were conducted on log transformed data and using the program INFOSTAT (InfoStatversión 2011; Di Rienzo et al., 2011). Individuals with positive residuals are considered to be in a better condition than individuals with negative residuals (Litzgus et al., 2008). Relationship between BCI and abundance of fungi (CFU) was analyzed with linear regression.

Fungi were found in 9 out of 13 cloacal samples (69.2%), 5 females out of 9 (55.6%) were positive for only one species of fungus, the others (4 out of 9 = 44.4%) swabbed positive for more than one fungus.The occurrence of multiple species of isolated fungi (more than two species) within a single host was uncommon, being presented in only one female sampled (1 out of 13). Different distributions of fungal occurrence were found on positive animals (Table 1), being: filamentous fungi (n=19 CFU), yeasts (n=7 CFU) and non-sporulating fungi (n=3 CFU).

Altogether, 11 genera, 14 species (including yeast and non sporulating fungi) and 29 CFU of fungi were isolated. *Aspergillus niger* (13.8%; n = 4 CFU; 3 out of 13 females sampled) was the most commonly occurring isolated organism followed by *Alternariaalternata* (10.3%; n = 3 CFU).*Fusariumredolens*,*Chaetophoma sp.* and *Scopulariopsischartarum* were represented by 6.9% (n = 2 CFU) each; while *Acremoniumfusidioides*, *A. strictum*,*Cirrenaliadonnae*,*Epicoccumpurpurascens*,*Trichodermaharzianum* and *Zygosporiumechinosporum* were represented by one CFU (3.4%; n = 1 CFU) in samples. Other isolated organisms, including hyaline *Mycelia sterilia* and *Dematiaceous bulbs* were detected.

From the total species found (n = 14) when grouped by their econutritional type the results showed that biotrophic-saprotrophs fungi had higher abundances percentages than biotrophic-pathogens fungi, yeasts and non-sporulating fungi respectively (Table 1). Thetotal fungi abundance and the groups of pathogen fungi and saprotrophs fungi were not related with apoor body condition of *C. latirostris* females (p values >0.77).

As was observed inthis study *A. niger* also have been isolated from skin and intestinal contents of *Crocodylus porosus*, *Osteolaemustetraspis* and *Alligator mississippiensis* (Buenviaje et al., 1994; Hibberd et al., 1996); while *A.alternata* was isolated from *A.mississippiensis* skin and as normal constituent of cutaneous mycobiota in other reptiles (Paré et al., 2003). Pathogens like *A. niger* and some *Fusarium* species has worldwide distributions and could be found on different types of substrates (Domsch et al., 1993) and had been implicated repeatedly in crocodilian severe mycotic pneumonias (Frelier et al., 1985; Ladds, 2009).We can account *F. redolens* as constituent of cloacal mycobiota on two of the females captured and it is widespread in the temperate zone and also tropical region (Domsch et al., 1993).Some species of genus *Fusarium* contain elaborate toxins (Richard, 1990)and were listed in eggs membranes of *C. latirostris* (unpublished data). Future studies could evaluate the effects of these fungi on some aspects of the reproductive traits (i.e. hatching success and hatchlings survival rate) and mycosis affecting animal health.

Our results also show the appearance of *S.chartarum.*In this genus most of them are soil fungi, and a few species become human-pathogenic (Domsch et al., 1993).*Chaetophomasp.* was found andthis is a coelomycete known mainly from tropical and temperate regions (Sutton, 1973); it was found in stems and roots of *C. latirostris* nests (unpublished data). Other fungi isolated were *E. purpurascens*, *CurvularialunataAcremoniumstrictum,A. fusidioides*and*Trichodermaharzianum*; these were found on previous works on skin and intestinal contents of *O. tetraspis* and *A. mississippiensis* (Huchzermeyer et al., 2003) andas a causative agent of a fatal diffuse granulomatous pneumonia and focal necrotizing hepatitis in spectacled caimans (*Caiman crocodilus*) (Trevino 1972). *Trichoderma*is generally regarded as low-grade opportunistic pathogens (Jacobson, 2007). In this study was made the first report of hyaline *M.sterilia* and *D. bulbs* on crocodilians.

There is no record for *Z. echinosporum* and *C. donnae* on crocodiles and/or alligators. With our results we added these biotrophic - saprotrophs fungi as new records for Crocodilian and extent the fungi known distribution to Argentina. *Zygosporiumechinosporum* was cited for Brazil (Gusmão et al., 2001) and Peru (Matsushima, 1993) and *C. donnae* was found for first time in Canada (Sutton, 1973) and there are some works recording this species in China (Zhao and Liu, 2005). Yeast (*Rhodotorula* sp.) found here was also isolated from lungs, liver, and kidneys of turtles, lizards, snake, and are highly prevalent in the gut of all reptiles (Enweani et al., 1997; Reavillet al., 2004).

These fungi which are not host specific to crocodilians can be isolated from a wide range of animals with and without signs of disease (Mitchell and Tully, 2009). The species of fungi/frequency in the females sampled were not related to their BCI. We cannot used these individual values to explain the ten genera of fungi found on one of these females and/or if the number of species/fungi load could be consequence of a good or a poor animal body condition.Probably,*C. latirostris* femalesacquired fungal contaminants in the cloaca by contact with feces of other contaminated animals, or during defecation by contact with contaminated substrate which was accumulated on the exterior of the cloaca and/or during oviposition by contact with nest substrate (Phillot et al., 2002). The distribution of the same fungal isolates in different females hosts suggest that reptiles may act as facultative animal carriers for fungi and yeast in their cloacae(Nardoni et al., 2008); and the presence of biotrophic – pathogen isolates could be usedas a tool to identify potential fungal pathogens which could exert a negative effect on wild caimans.

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**Table 1.** Mycobiota obtained from wild females of*Caiman latirostris* (swabbed positive for fungi). List of fungispecies,percentageoccurrenceof CFU(colonies forming-units) ((*ni CFU/total CFU sampled)\*100*) andpartial percentage of econutritional types (E.T) of each fungi species identified.

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| --- | --- | --- | --- | --- |
|  | **Econutritional types** | **Species of fungi** | **% Ocurrence of CFU** | **Partial% of E.T** |
| **Filamentous fungi** | **Biotrophic - pathogens** | *Aspergillus niger* | 13.8 | 27.6 |
| *Acremoniumfusidioides* | 3.4 |
| *Acremoniumstrictum* | 3.4 |
| *Fusariumredolens* | 6.9 |
| **Biotrophic - saprotrophs** | *Alternariaalternata* | 10.3 | 37.9 |
| *Cirrenaliadonnae* | 3.4 |
| *Chaetophoma sp.* | 6.9 |
| *Epicoccumpurpurascens* | 3.4 |
| *Scopulariopsischartarum* | 6.9 |
| *Trichodermaharzianum* | 3.4 |
| *Zygosporiumechinosporum* | 3.4 |
|  | **Yeasts** | *Rhodotorula sp.* | 24.1 | 24.1 |
|  | **Non – Sporulating fungi** | *Hyaline MiceliaSterilia* | 3.45 | 10.34 |
|  | *Dematiaceous bulbs* | 6.90 |