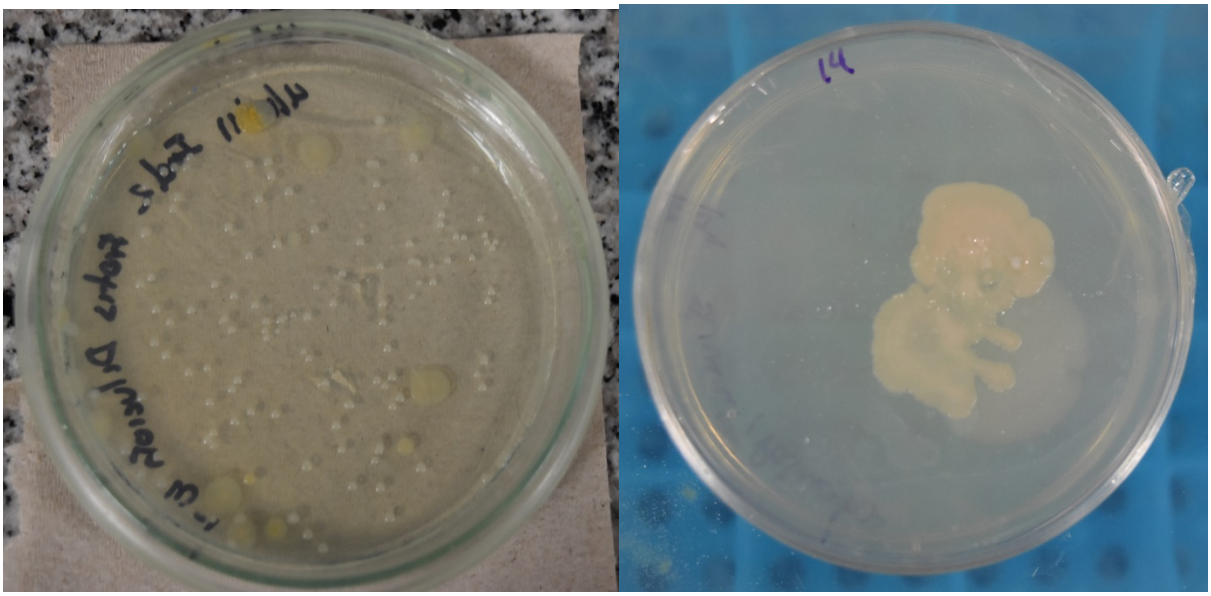
Project Update: March 2018

To describe the skin fungi community, we captured 10 tadpoles, nine juveniles and three adults of *Rheobates palmatus* (Aromobatidae) and one juvenile of *Dendropsophus molitor* (previously known as *D. labialis*; Hylidae). For all procedures, we used sterile materials.

To isolate skin fungi, each individual was rinsed twice with sterile water (50 mL) to remove transient microbiota. We used two approaches to recover the highest proportion of fungi: 1) We did impressions of each individual in Potato Dextrose Agar (PDA), and incubated the petri dish at 25 °C for 8 days. Then we transferred each morphotype to a new petri dish until obtain pure cultures. 2) We swabbed the frog's left, right and ventral surfaces using a synthetic rayon swab (MWE 100). Samples were preserved in a 2 ml tube with 0.85% saline solution at -20 °C until processed. To isolate fungal colonies, we plated out dilutions of 1:10, 1:100 and 1:1000 of the saline solution where swabs were stored on PDA media with antibiotic (Rifampicin), Malt Extract Agar and Sabouraud Agar. Petri dishes were incubated at 25 °C for 8t days. Finally, we isolated each morphotype and plated them in PDA with antibiotic (Rifampicin) and incubated at 25°C.

For strains identification, we employed 1) macroscopic characteristics, i.e.,

appearance, obverse and reverse colour of the colony, and size of the colony, 2) microscopic characteristics observed under magnification, i.e., colour, septation, branching. To date, we have isolated 26 fungal morphotypes. We are in the process of identification through sequencing ITS2 rRNA gene (DNA barcode) with the primers ITS1 and ITS4. In the table below, we summarized the number of isolates per species and life stages.



To determine infection status by *Batrachochytrium dendrobatidis* (Bd), each animal was swabbed by running a synthetic rayon swab (MWE 100) ten times over the ventral surface, the inner thigh area and the plantar surface of the hind feet webbing for a total of 50 strokes. Swabs were stored dry at -20 °C. Presence of Bd will be diagnosed and quantified using quantitative PCR (Real-Time PCR). Each individual was swabbed twice, one swab to test for Bd and one swab to isolate skin fungi.

Species	**Frog ID**	**Life stage**	**Fungal morphotype**
R. palmatus	1	Tadpole	1,2,3,4,5,7,11
R. palmatus	2	Tadpole	1,2,4,5,6,7,8,9,10,11
R. palmatus	3	Tadpole	2,5,6,11
R. palmatus	4	Tadpole	1,2,4,5,8,11,15
R. palmatus	5	Tadpole	1,2,7,11,14
R. palmatus	6	Tadpole	2,4,5,6,11,12
R. palmatus	7	Tadpole	1,2,4,5,8,11,17
R. palmatus	8	Tadpole	1,2,5,8,11
R. palmatus	9	Juvenile	2,4,5,8,11,13,
R. palmatus	10	Juvenile	2,5,8,11,19,20
R. palmatus	11	Tadpole	2,5,8,11
R. palmatus	12	Tadpole	2,4,6,7,11,19
R. palmatus	13	Juvenile	2,5,11,26

R. palmatus	14	Juvenile	2,4,5,6,7,11,19
R. palmatus	15	Adult	1,2,4,5,6,7,12,22,23,24
D. molitor	16	Juvenile	2,4,5,11,23,25
R. palmatus	17	Juvenile	2,3,4,5,6,8,19
R. palmatus	18	Juvenile	2,6,11,19
R. palmatus	19	Adult	2,4,8,11,19
R. palmatus	20	Adult	1,2,4,5,6,7,11
R. palmatus	21	Juvenile	2,4,6,8,11
R. palmatus	22	Juvenile	2,4,6,8,11,24
R. palmatus	23	Juvenile	2,4,6,7,11