

EVALUATION OF BACTERIAL INTERACTION WITH SKIN OF *Brachycephalus ephippium*

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Abstract: The skin of amphibians plays a key role in their homeostasis, but also favors the growth of microorganisms that usually live in a delicate balance on the skin surface. Once this balance is disrupted, some microorganisms can become opportunistic and potentially pathogenic and can contribute to amphibian population decline. *Brachycephalus ephippium* is a tiny frog, member of *Brachycephalidae* family, with diurnal habits and inhabiting the Atlantic Forest in southeastern Brazil. One of its main characteristic is the brilliant yellow color and the occurrence of tetrodotoxin in its skin, liver and ovaries. This work aims to evaluate the interaction between two species of bacteria (*Escherichia coli* and *Citrobacter freundii*) and *Brachycephalus ephippium* skin performing an *in vivo* experimental infection, and analyzing the results by means of morphological studies. In this *in vivo* model, overnight cultures at 37°C of *C. freundii* and *E. coli* were used to experimentally infect a group of four animals (for each bacteria specie) for five days. After sacrifice, skin samples were collected from dorsal and ventral regions, preparations were morphologically analyzed by light (LM) and electron microscopy (EM), and compared with fragments from a two non-infected animals. Results clearly show that *C. freundii* did not adhere to the *B. ephippium* skin. *E. coli*, on the other hand, presented a weak adherence to the skin, and this adherence was slightly stronger on fragment collected from ventral area. EM showed an intimate adherence of *E. coli*, but no signs of invasiveness were found. EM also showed that weak bacterial interactions as well as presence of biofilm-like structure were also frequent. LM analysis also showed differences in the aspect of granular (or poison) glands between infected and non-infected animals. Less granular (poison) content was observed in glands from fragments collected from dorsal regions of animals infected with *E. coli*. This study clearly shows that *C. freundii* is unable to colonize *B. ephippium*. The difference in content volume observed in granular glands between infected and non-infected animals suggests a reactive glandular discharge of the skin against the bacterial infection. The contents of the glands will be further analyzed by histochemistry.

Key-words: *Escherichia coli*, *Citrobacter freundii*, *Brachycephalus ephippium*, bacterial interaction, amphibian skin

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