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CYCLE XXIX

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Ecophysiological studies on the cutaneous apparatus of Anura: structure and ultrastructure of syncytial glands in *Agalychnis callidryas*

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Background. The cutaneous apparatus of Amphibia plays a key role in relationships between individuals and environment, contributing to many physiological processes. A relevant ecophysiological role in Anurans is played by exocrine serous glands, composed of an epidermal duct, a subepidermic part and an intradermal complex including a myoepithelium under orthosympathic control and a secretory unit. This unit with a typical syncytial structure produces active compounds involved in the paracrine regulation of cutaneous microenvironments, but also in chemical defence against infective agents and predators. Conditions leading to a deregulation of serous glands may contribute to decline of Anuran populations, because of their incomplete adaptation to subaerial environment and major physiological changes occurring before and after metamorphosis.

Scope and methods. Within research projects aimed to protect Anuran populations by examining physiological and environmental causes of their decline, structural and ultrastructural studies were conducted on syncytial cutaneous glands of *Agalychnis callidryas* (Anura, Hylidae, Phyllomedusinae), an Anuran species listed under "least concern" on the IUCN Red List of Threatened Species (2016). This species, also known as "red eyed tree frog", native of tropical rain forests of Central America, may represent a model species because of its secretory and behavioural mechanisms to preserve transcutaneous water balance. The studies involving optical and electron microscopy were conducted in collaboration with the research group led by Professor Giovanni Delfino (Department of Biology, University of Florence, Italy), a renowned international expert in studies on the cutaneous apparatus of Amphibia.

Results. Syncytial cutaneous glands specialized for the production of lipids were identified for the first time in larvae and adults of *A. callidryas*. In the genus *Phyllomedusa* the hydrophobic secrete is involved in "wiping behaviour", a series of stereotyped movements distributing secretions on the body surface to prevent transcutaneous dehydration. Syncytial glands belonging to the classic serous type were also described: they produced secretory granules characterized by periodic substructures during their maturation. These results allow to include this species among those phylogenetically related or unrelated converging in production of repetitive substructures in their secretory granules. The results also showed the presence of immature serous and lipid glands in premetamorphic larvae, supporting the hypothesis of an early appearance and differentiation of these glands in Anurans. In *A. callidryas* adults the lipid glands are similar to those described in *Phyllomedusa* and associated to wiping behaviour: they could therefore represent an anatomical preadaptation to this complex set of stereotyped movements. In serous glands, the maturation of secretory granules was also examined by Fourier transform analysis: the results show that granules may represent active "storage organelles" surrounded by a membrane derived from the Golgi apparatus: the microenvironment within these

organelles could be changed chemically or physically in order to modify the condensation of secrete and ultimately its biological activity.

Conclusions and perspectives. The identification of cutaneous lipid glands in a species apparently not performing the wiping behaviour strongly suggests to extend investigations to other species of tree-living frogs. Because of their role in preventing dehydration, studies on these glands are relevant for ecophysiology and conservation of threatened Anuran species. Studies on cutaneous glands producing secretory granules with repetitive substructures are also very interesting because these structures may confer flexibility and adaptability to the secrete, that could be entirely discharged as a defence against predators or released in discrete amounts for cell signalling and other regulatory processes.