Organization of adhesive toe pad of *Philautus annandalii* (Boulenger, 1906): An ultrastructural and immunohistochemical study

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph. D.) IN SCIENCE OF VIDYASAGAR UNIVERSITY

SAURABH CHAKRABORTI

DEPARTMENT OF ZOOLOGY VIDYASAGAR UNIVERSITY MIDNAPORE (WEST) – 721102 WEST BENGAL, INDIA

2013

SYNOPSIS

This present study describes the organization of adhesive toe pads of *Philautus annandalii* (Boulenger, 1906), a tiny tree frog found in the montane shrubland of Eastern Himalayas. By means of these expanded tips of digits they adhere to the plant substratum of their arboreal habitat, often in near vertical situation, while performing the all the socio-biological activities such as reproduction, prey capture and predator avoidance etc. To decipher the possible mechanism of adhesion by this frog, underlying structural peculiarity of the entire toe pad, specifically its ventral epidermis, have been studied by using light microscopy and scanning – and transmission electron microscopy. Unlike the epidermis present in the dorsal aspect of the toe pad, the functional ventral toe pad epidermis shows multiple specializations. It is relatively thicker and composed of four rows of cells organized into three distinct layers. The outermost epithelial cells are the most specialized. They are mostly hexagonal and columnar in shape and separated from the adjacent cells by wide mucous-filled channels. Randomly placed mucous pores are also present in-between these cells. These cells possess numerous peg-like micro projections on their surface, which are reinforced internally by thick bundles of keratin filaments. These are the mechano-functional ultrastructural subunits of the toe pads - generating frictional forces as well as interlocking mechanically with the rough plant surfaces both essential for successful adhesion. The cytoplasm of the epithelial cells contains no organelles, but pleiomorphic nuclei and small mucous granules. The mucous seems to function as glue or matrix material in bundling of cytokeratin filaments. The intermediate epidermal cells are cuboidal and possess euchromatic nuclei and many cytoplasmic organelles, especially ribosomes. Like the outer epithelial cells, they also possess keratinized microprojections on their apical part, which are somewhat immature in appearance. When the outer layer is shed, the intermediate layer is ready with features of adhesion. These cells contain more keratin than the epidermal cells. The basal layer contains two rows of columnar cells, each having thin keratin bundles and usual cell organelles in addition to a euchromatic nucleus. The toe pad dermis is rich in blood vessels, collagen bundles and mucous glands – the ducts of which open mainly at the intermediate – basal layer junction of the toe pad epidermis. During adhesion, when the toe pads are pressed against the substratum, the secretion of these mucous glands is spreaded uniformly over the entire epidermal surface through the deep grooves of the outer cells and thus aid in the process of wet adhesion by the toe pads, which is achieved by the combination of capillarity (surface tension forces) and viscosity forces (Stefan adhesion). No lipidic material is involved in the process adhesion as the epidermis show complete absence of such material by lipid specific histochemical staining, viz., oil-red O staining. Immunohistochemical and immunoblot studies reveal that the cytokeratin, present predominantly in the intermediate and basal layers of the toe pad epidermis, have the molecular mass ranges between 45-50 kDa and are suggestive of alpha-keratin in nature, as reported in anamniote epidermis.