UTTAR PRADESH JOURNAL OF ZOOLOGY

39(2): 65-68, 2019
ISSN: 0256-971X (P)

ULTRASTRUCTURE OF THE SKIN OF THE TREE FROG *Polypedates leucomystax*

A. J. IANGRAI[1*] AND R. N. K. HOOROO[2]

[1]Department of Zoology, Synod College, Shillong – 793002, Meghalaya, India.
[2]Department of Zoology, North Eastern Hill University, Shillong – 793022, Meghalaya, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Received: 23 February 2019
Accepted: 26 April 2019
Published: 18 May 2019

Original Research Article

ABSTRACT

Amphibians live in varied habitats. This fact probably explains the great morphofunctional diversity in their skins. Although the integument is the structural and functional interface between the organism and its environment, the morphological and complexity of amphibian skin is not fully understand. There is practically no information on the integument of the tree frog *Polypedates leucomystax* found in Cherrapunjee. Therefore, ultrastructure of the skin of the adult frog of *Polypedates leucomystax* was studied during the breeding phase with the help of transmission electron microscopy. Adult males and females of Polypedated leucomystax were collected from the study sites at Cherrapunjee. The epidermis consisted of four layers namely, stratum corneum, stratum granulosum, stratum spinosum and stratum germinativum. Desmosomes, ribosomes, tonofilaments, golgi bodies, vacuoles and collagen fibres were observed in the epidermal cells. The basement membrane composed of collagenous fibres separated the epidermis from the dermis. Pigment cells like pterinosomes, melanophores and iridophores were absorved and these pigment cells may work together to determine the varied skin colouration and patterns displayed in *Polypedates leucomystax* and may have important roles in adapting this frog to its environment.

Keywords: *Polypedates leucomystax;* skin; epidermis; dermis; pigment cells.

1. INTRODUCTION

Amphibians live in varied habitats. This fact probably explains the great morphofunctional diversity in their skins. Amphibian skin is morphologically, biochemically and physiologically complex organ, which performs a wide range of functions (chemical defense [1], ion transport [2] necessary for the amphibian's survival. There are several reports on details structure including its ultrastructure and functions [3,4,5,6,7,8]. Compared with other vertebrates, amphibian skin is quite permeable to water, ions and gases [9], though considerable variation occurs among different species and at different times and conditions in the same animal. Most amphibians are poorly adapted to a strictly terrestrial life. Amphibian skin interacts with biotic and abiotic factors in their environment, but it does not in general serve as a barrier to the inward or outward flow of water to and from amphibian body [10]. The skin of amphibian is generally described as being naked due to the absence of the covering of scales, feathers or hair characteristics of most other classes of vertebrates. Detailed macroscopic and histological studies of the amphibian integument by Rabl [11] and Elias and Shapiro [12] have shown that

**Corresponding author: Email: asorphi@gmail.com;*

the epidermis varies in thickness and may have projections or indentations and that there are elevations and thickenings of various kinds in the dermis, especially in anurans. Skin thickness and the number of epidermal skin layers vary in species specific manner. One species will also vary from another in the process of keratinization. These characteristics are related to environment – aquatic or terrestrial [13].

Amphibians have diverse pigmentary patterns that include spots, stripes and mottles of various colours that can provide them either a cryptic or aposomatic colouration in their environment. It is well known that skin colouration is brought about by the interaction of the three kinds of chromatophores – xanthophores (erythrophore), iridophores and melanophores [14,15,16,17,18,19]. Although the integument is the structural and functional interface between the organism and its environment, the morphological and functional complexity of amphibian skin is not fully understand [7]. There is practically no information on the integument of the tree frog *Polypedates leucomystax* found in Cherrapunjee, East Khasi Hills, Meghalaya, India. This frog is arboreal and come to the ephemeral pools only for breeding and hibernate during the winter. As far as the skin colour is concerned *Polypedates leucomystax* shows brownish to light brown-yellowish colouration. During the breeding phase, male skin pigmentation is darker as compared to the female, which shows lighter pigmentation. Keeping this in view a transmission electron microscopic study on the integument of *Polypedates leucomystax* was undertaken to have a clear understanding on the role of ultrastructural specialization, if any in the skin colour of *Polypedates leucomystax*.

2. METHODOLOGY

To study the untrastructure of the skin of *Polypedates leucomystax*, adult males and females were collected from Cherrapunjee, East Khasi Hills, Meghalaya, during the breeding season. The collected adult frogs were anesthesized and the sections of the skin from the dorsal and ventral parts of males and females were processed for transmission electron microscopic studies.

The skin sections of the dorsal and ventral parts taken from both male and female frogs during the breeding period were fixed in 2.5% gluteraldehyde (prepared from 0.1M Na-cacodylate buffer) at $4^0$0 for 2 hours and post fixed with 1% buffered Osmium tetraoxide (prepared in 0.1M Na-cacodylate buffer) at $4^0$0 for 1 hour. The samples were than dehydrated through acetone grades (30%, 40%, 50%, 60%, 70%, 80%, 90% &100%) and then cleared in propylene oxide. The samples were then infiltrated in a mixture of clearing agent propylene oxide and embedding medium. After infiltration the tissues were embedded in epoxy resins using beam capsules and blocks were prepared. Ultrathin sections (60 – 90nm) were cut with the help of ultratome (Ultratome V, LKB), stained with uranyl acetate and lead citrate and were examined with a Transmission electron microscope (JEM 100C x 11Joel) at an accelerating voltage of 80KV.

3. RESULTS

The electron micrograph of the skin of male and female of *Polypedates leucomystax* revealed that the epidermis consisted of four layers of epithelial cells namely stratum corneum, stratum granulosum, stratum spinosum and stratum germinativum. The stratum corneum, which is the outer most layer was thickened and keratinized and consisted of a single layer of flattened cells. Protuberances on the outer surface of the epidermis were also observed (Figs. 1 & 2). The keratinized stratum cormeum was separated from the underlying stratum granulosum by intercellular spaces. In the stratum granulosum some cells were observed to be devoid of a nucleus. These cells had prepared to move to the stratum corneum, as stratum corneum sloughed or shed periodically and have to be replaced. Tonofilamnets and desmosomes were seen in the epidermal layers (Figs. 1 & 2). Following the stratum granulosum was the stratum spinosum and stratum germinativum. Cells of different shapes with prominent nucleus were observed in the stratum spinosum and stratum germinativum of *Polypedates leucomystax* (Fig. 3). These cells with prominent nucleus were joined together by desmosomes. Cell organelles like ribosomes, tonofilaments and mitochondria were present in the stratum germinativum.

Located beneath the epidermis was the dermis, which was separated from the epidermis by the basement membrane, composed by collagenous fibres (Fig. 4). Transmission electron microscopy revealed that the dermis of *Polypedates leucomystax* consisted of two layers, the stratum spongiosum and the underlying stratum compactum. In the stratum spongiosum pigment cells like pterinosomes and melanophores were present (Figs. 4, 5 & 6). Higher magnification of the pterinosomes showed that it was arranged in concentric layers (Fig. 7). Iridophores or reflecting platelets were also observed in the dermis. The stratum compactum includes a collagenous fibres arranged in a series of alternating layers (Fig. 8).

4. DISCUSSION

The epidermis of *Polypedates leucomystax* consists of a number of layers, which are similar to the other

amphibians [20,21]. Since *Polypedates leucomystax* is adapted to arboreal mode of life, the outer most layer of the epidermis, the keratinized stratum corneum with little recognizable ultrastructure may be related to their terrestrial adaptation, as it has been suggested that keratinizaton of body epidermis may be a crucial adaptation for terrestrial life [22]). *Polypedates leucomystax* leading an arboreal life style, visit the ephemeral pools solely during the breeding period. Hence, the integument as an adaptable organ system may play an important role in both aquatic and terrestrial mode of life as it has been reported that the skin of amphibian performs several functions such as mechanical protection [23], water absorption [24, 25] and respiration [7].

As observed in the stratum granulosum the epithelial cells were devoid of nucleus suggesting that these cells prepared to move from the stratum germinativum to the stratum corneum, to be replaced and shed periodically. It was observed that the epidermal cells with nucleus in the stratum germinativum moved to the upper layer i.e., stratum spinosum and later into the stratum granulosum and finally move towards the stratum corneum. Thus, it may be suggested that the epidermis participates in the process of keratinization. It has also been reported that in the epidermis of amphibians, cells from the germinativum layer move into the intermediate spinosum layer and later differentiate into cells of the replacement layer, the latter eventually replaces the external corneous layer during molt [26,27]. Presence of tonofilaments in the epidermal cells of *Polypedates leucomystax* suggests that they provide a mechanical support to the skin. Desmosomes observed between the adjacent epidermal cells permit surface shedding by their breakdown, since it has been suggested that desmosomes may ultimately be degraded by lysosomal enzymes [28].

Located beneath the epidermis is the dermis containing chromatophores or pigment cells. During the present study, pigment cells like xanthophores, melanophores and iridophores were observed. The xanthophores containing pterinosomes as pigmentary organelles imparts red, orange and yellow colour to the frog [29]. Melanophore located in the lower layers of the dermis imparts black or brown to the organism. The shining appearance of the skin in *Polypedates leucomystax* might be due to the presence of iridophores in the integument as it has been suggested that these iridophores may play a role in reflection, diffraction and other optical phenomena [30]. Similarly, [31] reported that skin colouration in the frog *Bokermannohyla alvarengai* is brought about by the arrangement of the iridophores. It is to be noted in this context that many vertebrates exhibit complex skin pigmentation patterns achieved by differential distribution of three pigment cell (chromatophores) types – xanthophores, melanophores and iridophores [32]. The yellowish brown colour in the dorsum of *Polypedates leucomystax* may be derived from the relative positions occupied by different types of chromatophores. The observation on the variation in skin colour from yellowish to brownish in the morning and evening in *Polypedates leucomystax* indicates pronounced colour change. It may be mentioned here that many kinds of pigment cells provide patterns and contribute colour change through the mediation of a variety of mechanisms involving both physiological and morphological colour changes. These changes occur as adaptive responses to several environmental cues, principally background colouration and the presence or absence of light. In the present study it may be suggested that the chromatophores may work together to determine the varied skin colouration and patterns displayed by *Polypedates leucomystax*, and may have important roles in adapting this tree frog to its specific environment.

5. CONCLUSION

On the basis of the present findings it may be concluded that the ultrastructure of the skin of *Polypedates leucomystax* was similar to other amphibians. The epidermis was composed of four layers. The outermost layer the stratum corneum was keratinized which may be related to their terrestrial adaptation. In the dermis, pigment cells like pterinosomes, melanophores and iridophores were present. The colouration seen in this frog is the result of the work of these pigments found in the dermis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jekel AM, Saporito RA, Grant T. The relationship between poison frog chemical defenses and age, body size and sex. Frontiers in Zoology. 2015;12: 27.
2. Wu NC, Cramp RL, Franklin CE. Living with a leaky skin, upregulation of ion transport proteins during sloughing. J. Experimental Biology. 2017;220:2026–2035.
3. Catton WT. Cutaneous mechanoreceptors. In: Frog neurobiology (Eds., Llinas R, Precht W). Springer Verlag, Berlin. 1976;629-642.
4. Lindemann B, Voute C. The structure and function of the epidermis. In: Frog

neurobiology (Eds. Llinas R, Precht W). Springer Verlag, Berlin. 1976;169-210.
5. Fox H. Amphibian morphogenesis. The Humana Press, Clifton, New Jersey; 1984.
6. Delfino G. Ultrastructural aspects of venom secretion in anuran cutaneous glands. In: Handbook of Natural Toxins Reptile venoms and Toxins (Ed., Tu AT). Marcel Dekker, Inc. New York, Basel. 1991;5:777-801.
7. Duelman WE, Trueb L. Biology of amphibians. John Hopkins University Press, Baltmore and London; 1994.
8. Delfino G, Grach F, Malentacchi C, Nosi D. Ultrasytuctural evidence of serous gland polymorphism in the skin of the tungara frog *Engystomops pustulosus* (Anura: Leptodactylidae). Anatomical Record. 2015;298:1659–1667.
9. Bentley PJ, Baldwin GF. Comparison of transcutaneous permeability in skin of larval and adult salamanders. Am. J. Physiol. 1980;239:505-508.
10. Hadley NF. Surface waxes and integumentary permeability. Am. Sci. 1980;68:546-553.
11. Rabl H. Integument der Anamnier. In: Handbuch der Vergleichenden Anatomie der Wirbeltiere (Eds., Bolk LE, Goppert E, Kallius E, Lubosch W). Berlin. 1931;I: 271-374.
12. Elias H, Shapiro J. Histology of the skin of some toads and frogs. Amer. Mus. Novit. 1957;1819:1-27.
13. Bani G. La morfologia della pelle di anfibi: Confront tra alcuna specie a differenti abitudini di vita. Boll. Soc. Ital. Biol. Sper. 1982;58:625-631.
14. Bagnara JT, Hadley ME. Chromatophores and color change. Englewood Cliffs, New Jersey: Prentice-Hall, Inc.; 1973.
15. Oliphant LW. Epidermal xanthophores in a salamander. Can. J. Zool. 1973;51:1007-1009.
16. Bagnara JT, Frost SK, Matsumoto J. On the development of pigment patterns in amphibians. Am. Zool. 1978;18:301-312.
17. Bagnara JT, Turner Jr. WA, Othstein JR, Ferris W, Taylor JD. Chromatophore organellogenesis. Pigment Cell. 1979;4:13-27.
18. Frost SK, Robinson SJ. Pigment cell differentiation in the fire-bellied toad, *Bombina orientalis*. 1. Structural, chemical and physical aspects of the adult pigment pattern. J. Morphol. 1984;179:229-242.
19. Taylor JD. Does the introduction of a new player, the endoplasmic reticulum, create more or less confusion in understanding the mechanism(s) of pigmentary organelle translocation? Pigment Cell Res. 1992;5:49-57.
20. Whitear M. Flask cells and epidermal dynamics in frog skin. J. Zool. Lond. 1975;175:107-109.
21. Blankemeyer JT, Shahin SH. Increase in gap junctions in frog skin epithelium. Proc. Okla. Acad. Sci. 1993;73:23-27.
22. Menon J, Gardner EE, Vail S. Developmental implications of differential effects of calcium in tail and body skin of anuran tadpoles. J. Morphology. 2000;244:31-43.
23. Fox H. The skin of amphibian. In: Biology of the integument 2 Vertebrates (Eds. Bereiter-Hahn J, Matoltsy AG, Richards KS). Springer-Verlag, Berlin. 1986;78-135.
24. Hoffman J, Katz U. Elevated plasma osmotic concentration stimulates water absorption response in a toad. J. E p. Zool. 1999;284:168-173.
25. Sullivan PA, Hoff KVS, Hillyard SD. Effects of anion substitution on hydration behaviour and water uptake of the red-spotted toad, *Bufo punctatus*: Is there an anion paradox in amphibian skin? Chem. Senses. 2000;25:167-172.
26. Budtz PE, Larsen LO. Structure of the toad epidermis during the moulting cycle. I. Light microscopic observations in *Bufo bufo* (L.). Z. Zellforsch. 1973;144:353-368.
27. Budtz PE, Larsen LO. Structure of the toad epidermis during the moulting cycle. II. Light microscopic observations in *Bufo bufo* (L.). Cell Tissues Res. 1975;159:459-483.
28. Douglas WHJ, Ripley RC, Ellis RA. Enzymatic digestion of desmosome and hemidesmosome plaques performed on ultrathin sections. J. Cell Biol. 1970;44:211-215.
29. Bagnara JT. Cytology and cytophysiology non-melanophore pigment cells. Int. Rev. Cytol. 1966;20:173-205.
30. Menter DG, Obika M, Tchen TT, Taylor JD. Leucophores and iridophores of *Fundulus heteroclitus*: Biophysical and ultrastructural properties. J. Morph. 1979;160:103-119.
31. Centeno FC, Antoniazzi MM, Andrade DV, Kodama RT, Sciani JM, Pimenta DC, Jared C. Anuran skin and basking behaviour: the case of the tree frog *Bokermannohyla alvarengai* (Bogermann, 1956). J. Morphol. 2015;276:1172–1182.
32. Fernandez PJ, Bagnara JT. Observations on the development of unusual melanization of leopard frog ventral skin. J. Morphology. 1993;216:9-15.