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ULTRASTRUCTURAL STUDY OF THE LATERAL LINE NEUROMASTS IN TADPOLES OF SAUDI *BUFO DHUFARENSIS* AND *RANA RIDIBUNDA*

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Abstract

Neuromast (hair cells) structure in *Bufo dhufarensis* and *Rana ridibunda* larvae was observed by Scanning Electron Microscopy (SEM). Neuromasts were found arranged in one well-defined line in the head, body, and tail regions forming the lateral line and also found haphazardly scattered in most of the body parts. Their number was significantly high in the head region, and then it gradually decreased along the posterior end of the body. The structure of neuromasts in these three regions was basically similar for each species. In *Rana*, neuromasts were found few in number, either spherical or oval in shape lacking hair-like structure except in the tail region where hair cells were found. While in *Bufo*, neuromasts were numerous. Long kinocilia and many stereocilia were found in the neuromasts. Kinocilia were either solitary or in clusters. In addition to the main functions of the neuromasts we discovered a new function which was not found in previous researches, neuromasts were also used to remove any attached object on the tadpole's skin, by directing the kinocilium to the object thing and rolling onto it then detaching it outwards.

Key words: Saudi Arabia, SEM, *Bufo dhufarensis*, *Rana ridibunda,* hair cells,

Introduction

Neuromasts (sensory cells) are lateral line organs that form superficial sensory organs in amphibians and fish (Dijkgraaff, 1989; Northcutt, 1992). They are units of sensory hair cells with a ring of support cells surrounding them. The neuromasts detect water movements and facilitate schooling, prey capture and predator avoidance (Dijkgraaff, 1989). It was found that superficial neuromasts facilitate non-visual feeding (Yoshizawa *et al*, 2010; Yoshizawa and Jeffery, 2011; Sampson *et al*, 2013). Production of hair cells and supporting cells is either limited to a time period during development as in anuran tadpoles or continuous throughout life as in urodeles, depending on the species.

The lateral line system is a collection of epidermal sense organs (mechanoreceptive neuromasts and electroreceptive ampullary organs) and their nerves, distributed over the head and along the body in many aquatic amphibians (Maklakov *et al*, 2003; Warkentin, 2005; Claas and Dean, 2006; Fritzsch *et al*, 2006).

Neuromasts are found just on the skin surface. Each neuromast contains up to hundreds of mechanosensory hair cells that are surrounded by support cells. Hair cells function as directional sensors that convey information to the brain for amphibians to orient themselves relative to a water current (rheotaxis) (Suli *et al*, 2012), hold a stationary position in a stream, capture prey, avoid predators, and communicate with intraspecifics (Wada *et al*, 2013).

The ciliary bundle of each hair cell contains one long cilium (kinocilium) and a cluster of shorter cilia (stereocilia) (Hama, 1965; Jande, 1966). The ciliary bundles of the hair cells are embedded in a gelatinous cupula. Hair cells are activated when water flows past the skin surface, causing the cupula to move, thus causing the cilia to bend. The neural response of each hair cell is proportional to both the degree of cilia dis-

placement and the direction in which the stereocilia are displaced relative to the eccentrically placed kinocilium of each hair cell. The fine structure of this type of sense organ was examined with the electron microscope. Dijkgraaf (1963) and Shelton (1970) have studied the fine structure of the neuromast of *Xenopus,* while Jande (1966) investigated the neuromast of the *Rana* tadpole. Later studies of hair cell morphogenesis in *Rana catesbeiana* (Lewis and Li, 1973; Li and Lewis 1974) were based on *ontogeny:* all hair cells in that frog initially are formed without kinociliary bulbs; the bulbs develop gradually on certain hair cells as they mature, suggesting that the bulbed kinocilium is derived relative to the unbulbed kinocilium (Lewis 1981; Lee and Park, 2008). It was also found that neuromasts could be exposed to loss due to high concentration of cisplatin (Giari *et al*, 2012).

In this study ultrastructure of neuromasts of Saudi tadpoles were described. Complement these data by adding extra taxonomy to tadpoles of *Bufo dhufarensis* and *Rana Ridibunda*, by Scanning Electron Microscope.

Material and Methods

Manipulations: Fertilized eggs from couples of the available Saudi anurans species, of Dhufar toad *Bufo dhufarensis* (Al-Derayya Village, Riyadh) and frog *Rana ridibunda* (Al Hasa Region) were collected. After hatching the larvae were fed on a good meal of boiled spinach daily. The experimental stages were 49 in *Bufo* and 41 in *Rana* were selected according to the normal table (Sedra and Michael, 1961). Both stages are similar in characters and developmental rate of growth.

Experimental design: A number not less than 15 tadpoles were anaesthetized using 1:10000 MS-222 in distilled water for light microscopy investigation for the detection of the selected stage and the counts of neuromasts/mm using graduated eye piece lens. Investigation of neuromast was focused in three definite regions of the body where the cells were most abundant, the post-orbital region, the lateral line in the mid-body region and lateral line in the mid-tail region.

Scanning Electron Microscopy: These specimens were fixed in a 2-3% glutaraldehyde solution for 3-4 hrs at room temperature, followed by three 15 min washes in 0.1 M phosphate buffer. Then they were dehydrated in a graded ethanol series as follows: 35%, 50%, 70%, 80%, 95%, three changes at 100%, for 15 min each and a final 5 min wash in acetone 100%. Specimens were critical point dried in CO_2, mounted on aluminium stubs and sputter coated with gold. Features of neuromast cells were examined and photographed using a scanning electron microscope, attached to a computer.

Quantitative Analysis: Statistical analysis was performed using ANOVA and Student's two-tailed *t*-test (Flower and Cohen, 1997).

Results

In both species, the lateral line system having neuromasts was superficial lines on the epidermis. The main lines were bilaterally arranged in the head region and body. In the head region they were as follows: the supra-orbital line, the infra-orbital line, the post-orbital line and the oral line. The quantitative results were focusing on the post-orbital lateral line. But, there was also lateral line along the body axis reaching the tail region. In addition, more neurmasts were distributed haphazardly on the head and body. Neuromasts has kinocilia projecting from its apical surface. It has a group of sensory stereocilia surrounding the kinocilia. The non-sensory supporting cell was the base of stereocilia and kinocilia. Epidermis of *Rana* showed few number of neuromasts that were scattered along the body, but showed more abundances in the post-orbital region (15.6±0.2 neuromasts/mm) than the lateral line of the body and the tail regions (9.3±1.2 & 7.8±0.7 neuromasts/mm respectively) (Tab. 1). Neuromasts found in the post-orbital and body regions were protruded from the epidermal cells and lacked hair cells, they had different structures either

lobulated (Fig. 1a) or spherical (Fig. 1b). However, the neuromasts found in the lateral line of the tail region had hair cells that had a special structure. Most of them were found with fused hair cells. The hair cells of the same neuromast were either fused at their loose ends forming one long kinocilium (Fig. 2a), the hair cells of adjacent neuromast cells were fused together forming common kinocilia (Fig. 2b), hair cells were fused at their base (Fig. 2c) or hair cells formed quadruplet clusters (Fig. 2d).

Table 1: Mean ± S.E. of distribution of neuromasts along different body parts tadpole larvae of *Rana ridibunda* and *Bufo dhufarensis*

No. of neuromasts /mm Species	Post-orbital region	Lateral line of body region	Lateral line of tail region
Rana ridibunda	15.6 ± 0.2*	9.3 ± 1.2*	7.8 ± 0.7
Bufo dhufarensis	51.9 ± 0.3*	39.9 ± 0.0*	30.1 ± 0.1

$p<0.05$ vs. lateral line in tail region

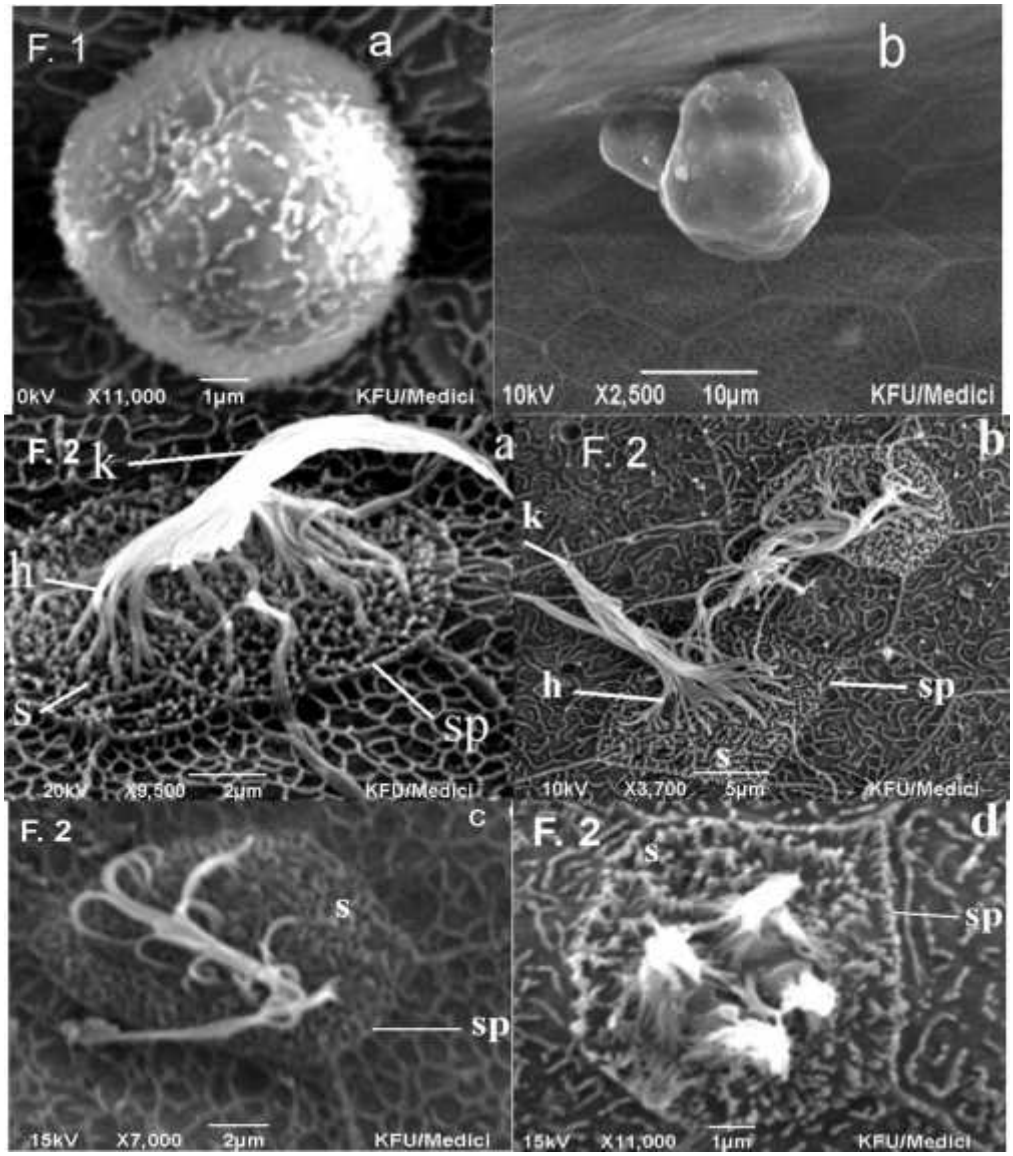

Fig. 1: SEM showing epidermis of post-orbital region of *Rana Ridibunda* with few neuromasts "hair cells". a) spherical and b) lobulated neuromasts.

Fig. 2: SEM showing different shapes of neuromasts (hair cells) in lateral line of tail region in *R. ridibunda*. a) Fusion as single kinocilium (k) within same neuromast (sp). b) Fusion of hair cells (h) within two adjacent neuromasts. c) Fusion of hair cells forming single horizontal cilium. d) Fusion of hair cells within the same neuromast forming quadruplets clusters of kinocilia. Stereocilia (s) short cilia.

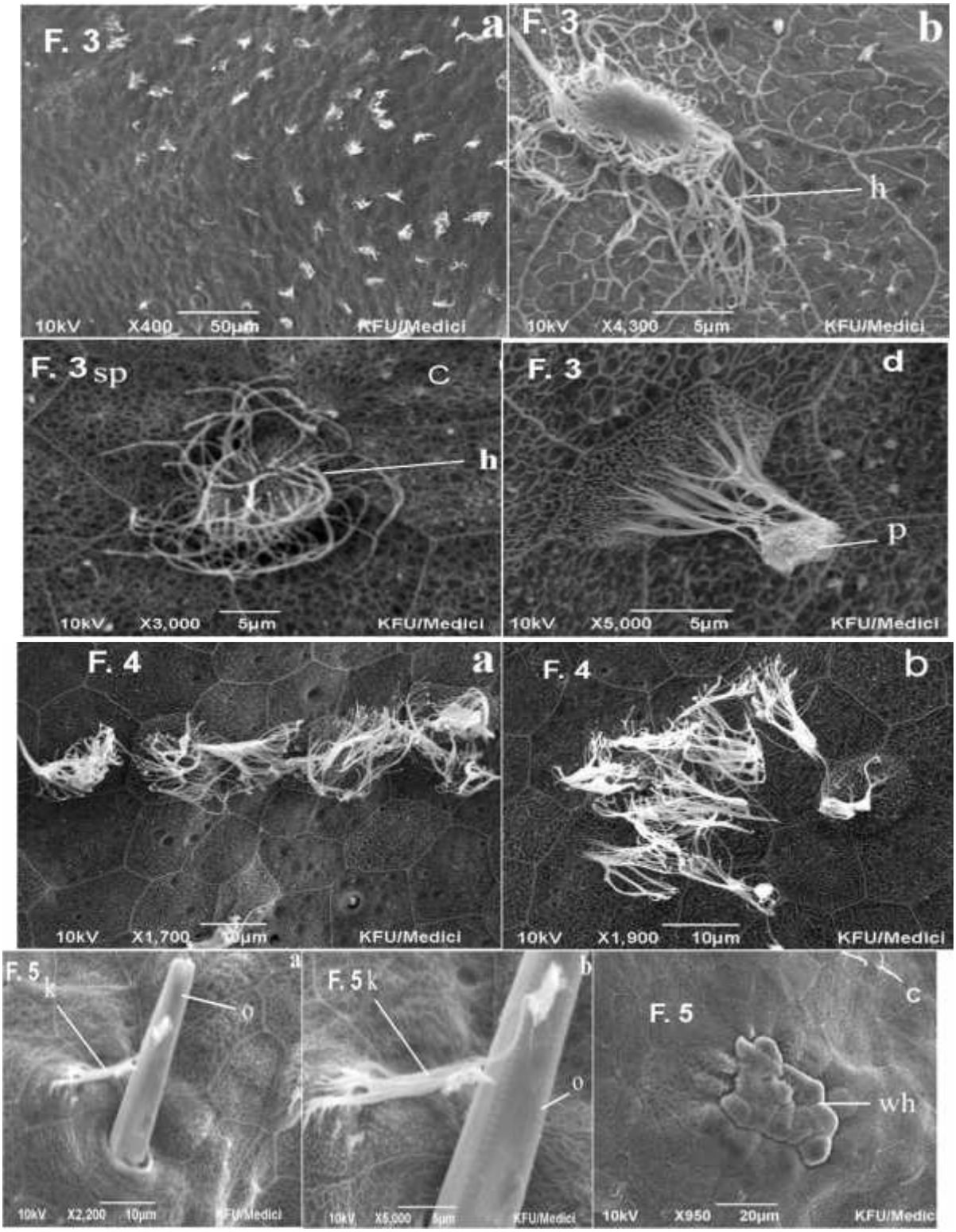

Fig. 3: SEM showing a) epidermis of post-orbital regions of *Bufo dhufarensis* with scatterd neuromasts. b) & c) neuromasts with free hair cells (h) directed according to water movement. (d) Hair cells moved towards food particles (p).

Fig. 4: SEM showing distributions of lateral line neuromasts in mid-tail region of tadpoles of *B. dhufarensis.* a) distribution in one line b) distribution in groups.

Fig. 5: SEM showing additional functions of kinocilium (k) in *Bufo dhufarensis*. a) Kinocilium with its hair cells extended and surrounding foreign object (o). b) Enlarged part of kinocilium rolling towards foreign object. c) Wound injury has been healed (wh).

The epidermis of *B. dhufarensis* showing numerous neuromasts (hair cells) which were distributed all over the body of the tadpole (Fig. 3a). Their number was much more than *Rana sp*. (Tab.1). Neuromasts number and shapes were varied according of their locations on the body. The post-orbital region had the maximum number of neuromasts, where it reached to 51.9 ± 0.3 neuromasts/mm. Furthermore, their number decreased within the lateral line of the body and tail. It was less in the mid-body region and least in the lateral line of the mid-tail region (39.9±0.0 & 30.1±0.1 neuromasts/ mm respectively). Their shapes varied from spherical to oval. Each one contained numerous scattered hair cells that appeared straight or curved depending on the movement of water (Fig. 3b & c) and/or the direction of food particles in the water (Fig. 3d)

Neuromast size was related to number of hair cells that they contained. The large neuromasts conained 40 or 50 hair cells; the smaller ones contained fewer than 15 hair cells. Neuromasts of the lateral line of the mid tail region were arranged in line (Fig. 4a) or in groups (Fig. 4b). Their shapes were similar to that of other parts. Neuromasts differ in size, shape, density and length of hair bundles according of their locations on different body regions.

A new discovery of a new function of the neuromasts was found in this work, which has not been previously discovered by other researches. Beside the known functions of the neuromasts which are detection of water vibrations, schooling, prey capturing and predetor avoidance .. etc. neuromasts were observed to detach objects that had pierced the tadpole's skin. It has been observed that kinocilium has directed towards an object which had pierced the tadpole's skin (Fig. 5a and b). The kinocilium rolled surrounding the object, then pulled the object outwards to detach it. After detaching the object the skin wound undergo healing (Fig. 5c).

Discussion

In the present findings, both species of amphibia had neuromasts, which were not equally distributed all over their bodies. The lateral line of the post-orbital region had significantly higher number of neuromasts. It gradually decreased along the anterior posterior axis of the larval body ($P<0.05$). This finding was in consistent with Dezfuli *et al*. (2009), where he found that the number of superficial neuromasts over the cavefish body was localized mainly in the head region, which was higher in number compared to the body region. However, the neuromasts in *Rana* was less in number in comparison with the *Bufo*. Nevertheless, some differences in the structure of neuromasts were observed between *Rana* and *Bufo*. In *Rana*, the neuromasts have been observed to be small in number and arranged in the same lateral lines, it was protruded from the squamous cells and lacking hair cells, except in the lateral line of the tail region. However in *Bufo*, the neuromasts were numerous with hair cells that were singly or in cluster. These differences in structure in both species agree with Wellenreuther *et al*. (2010) who suggested that interspecific differences in lateral line organs maybe a bi-product of selection for habitat divergence. Although we found that neuromasts were scattered all over their bodies, but they were condensed in the lateral line, which was arranged in three lines in the head region. This finding coincides with Nigam (1984), who described the lateral lines in the head region of *Bufo melanostictus* as follows: the supra-orbital line, the infra-orbital line, the post orbital line and the oral line.

Since it was observed in the present work that hair cells in *Bufo* have the ability to hold food particles (Fig. 3) and to detach objects piercing the tadpole's skin (Fig. 5), then we assume that these hair cells were secretory cells. Our assumption was based after Jorgensen and Flock (1973) who found that

the sensory hair cells in *Ambystoma* have the cytology of secretory cells.

Lewis (1981) found that the single cilium (kinocilium) of the hair cell has a bulb at its distal end. The present findings disagreed with what Lewis had found in anuran amphibia, no bulbs were found in the hair cells in both species. However, the present results agreed with Lewis (1981) results on urodeles where he found that the kinocilium of the urodele amphibian hair cell has no bulb. In addition, it was observed in *Bufo* that the hair cells of the hair cells of the same neuromast were fused at their loose ends forming one long kinocilium, the hair cells of adjacent neuromast cells were fused together forming common kinocilia, hair cells were fused at their base (Fig. 2c) or hair cells formed quadruplet clusters (Fig. 2d). This observation is in consistent with Nigam (1984), where he mentioned that a stable point of interest in the lateral line system in the tadpoles of *Bufo melanostictus* was that the neuromasts were found either solitary or in clusters of 2, 3 or 4. They were designed as singlets, doublets, triplets and quadruplets respectively. From our observations in the present study, we found a new function performed by neuromasts. Neuromasts can detach foreign objects pierced through the tadpole's skin by extending their hair cells towards the object and then pull it outwards. This new finding may support the notion of Uchiyama *et al*. (1991) who thought that neuromast systems have the function that is intermediate between touch and hearing, and is best described as a sense of touch-at-a-distance.

Collectively, the ability of the neuromast to tightly hold the foreign objects and the finding of Jorgensen and Flock (1973) that neuromasts have the cytology of secretion raised a new point of interest to investigate the nature of the adhesion ability of these cells.

Conclusion

The outcome findings showed that neuromasts were distributed in lateral lines all over the bodies of *Rana ridibunda* and *Bufo dhufarensis*. They were different in densities, high in the head region then gradually decreased to the posterior end of the larva.

Besides, neuromasts had different structures among both species. Finally, our work was the first to observe that neurmasts are able to detach objects pierced into the tadpole's skin. Further investigations and studies are required to explore more about the structure and the function of the amphibian neuromasts in general and their modification in secondarily aquatic amphibians in particular.

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