
Toe-pad morphology in White's tree frog, *Litoria caerulea* (Family Hylidae)

M. Nokhbatolfoghahai

Department of Biology, School of Sciences, Shiraz University, Iran
E-mail: nokhbeh@hotmail.com

Abstract

The aim of this study was to find any structural differences between the digital pads of forelimbs and hind limbs as well as more careful investigation of the internal and external structures of the toe-pad. In this study, pad morphology and cytology in *Litoria caerulea* is described using SEM, TEM and light microscopy. At the gross anatomical level, toe-pads in hind limbs were subdivided into medial and lateral parts by two large grooves. Semi-thin sections also showed that the toe-pad epidermis in hind limbs consisted of four layers with a cuboidal outermost layer, while the epidermis of forelimbs consisted of 3 layers with a columnar outermost layer. SEM study revealed two basic shapes of epidermal cells arranged very regularly across the surface of the pad: pentagonal and hexagonal. The pentagonal mainly occupied the most distal part of the toe. Three types of mucous-secreting pores were also seen in between the epithelial cells.

Keywords: Tree frog; *Litoria caerulea*; toe-pad; mucous pores; morphology

1. Introduction

The presence of enlarged toe-pads (=adhesive/digital pads) has been reported in different genera and species of several frog families to date: Hylidae [1-3], Microhylidae [4], Leptodactylidae [5], Hyperoliidae [6-7], Rhacophoridae [8-10], Ranidae [11], Centrolenidae [5], and Dendrobatidae [12]. These specialised expanded toe-pads serve to increase the surface area of the toes, which facilitates adhesion [13] and aid climbing ability on smooth, vertical surfaces or even sticking overhanging surfaces [1, 12, 14-19].

Despite the diversity of taxa in which toe-pads are found, there is a high degree of similarity in toe-pad cell structure among these unrelated frogs [3-5, 10-11, 13]. This similarity of toe-pad structure among different families of anurans has been interpreted by some authors as being a result of convergent evolution [5, 19-20]. In contrast, the morphological structure involved in toe pads like folds vary in position and shape, can be single or double etc. [11]. These structures can bear phylogenetic signal and are distinct among clades of frogs.

As presence of toe pads is linked to life in trees, shrubs or streams, there are many anuran species with different adaptations that have poorly-developed toe-pads or a total lack of toe-pad.

The surface morphologies of the digits of non-arboreal and semi-arboreal frogs may provide evidence of the possible stages leading to the development of the adhesive toe-pads of true tree frogs. Green [5] studied the surface structure of toe-pads in 29 species representing 17 genera of seven families of anurans, and found the transition of digital pad cell specialisation. The least amount of epidermal specialisation on the digits was found in the terrestrial toad (*Bufo americanus* and *Gastrophryne carolinensis*), with non-differentiated squamous shape of the epidermal cells; some differentiation of cell morphology showing slightly elevated cuboidal cells on the toe (*Rana clamitans*) to a further development of pad type was seen at the tip of the toe, although they were still not bounded by any grooves or distinct margins, to the highly developed digital pad cells (columnar) with grooves and channels in arboreal species and even in some non-arboreal species.

Several studies have been published on the structure of the digital pads, notably using scanning electron microscopy. In addition, many used transmission electron microscopy and it is somewhat surprising that there has been so little detailed light microscopy. Many studies also focused on the physiological aspects of toe-pad adhesion rather than concentrating on their histology.

Although the toe pad epithelium is, indeed, different from most areas of skin, the epithelium of

*Corresponding author

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subarticular tubercles, which also have an adhesive function, often has a very similar morphology. The cytological analysis of the toe-pad area by electron microscopy revealed modified and adhesive corresponding to those in surface morphology.

The greatest difference between the adhesive and non-adhesive epithelia concerned the outermost cell layer. Nevertheless, the surface cells were firmly interdigitated by finger-like processes with desmosomes at the lateral lower two-thirds and clearly separated from each other at their upper third (free apices). Different methods, including transmission electron microscopy and scanning electron microscopy [21] have revealed that the tops of the epithelial cells are not flat, but compose of a dense array, that they named nanopillars.

Green [5] reported two types of mucous pores classed as type I (simple) and type II (modified). Type II pores were found in most hylid species including *Litoria caerulea*.

The typically structured dermis contained numerous nerve fibers and was highly vascularized at the toe pad area and consisted bundles of collagen, and mucoid glands [3, 10, 22].

Among the literature on tree frog toe-pad, it is shown that *Litoria* is becoming a 'model organism' for the study of wet adhesion in amphibians, and though there are data on aspects of toe pad morphology on this species [19, 20, 23], a study that presents a more complete picture of toe pad morphology in this species is lacking.

The main purpose of this article is to study the surface and internal structure of toe-pads ultra-structurally and by semi-thin section in the species *Litoria caerulea* (White, 1790), Family Hylidae Rafinesque, (1815) with emphasis on dermal structure and some aspects of epidermal structure including mucous glands and pores. We will also look at differences between fore and hind toe pads, something that has not been attempted in any study, and that we will put the findings into the context of previous studies of toe pad morphology and ultrastructure.

2. Materials and methods

White's tree frogs (*Litoria caerulea*, family Hylidae) were purchased from commercial suppliers and maintained in glass vivaria at 20–24°C, using heat mats. The vivaria contained foliage, dishes of Cu-free fresh water to maintain a high humidity, branches on which the frogs could climb and sphagnum moss for the frogs to burrow into, all on a gravel base. They were fed on live house crickets dusted with a calcium balancer and multi-vitamin supplement (Nutrobal, purchased from Peregrine Live Foods, Ongar, Essex, England) twice weekly.

Toe pad study was carried out on three fully adult frogs with average snout-vent length 73 mm± 1.2 mm. I used the largest toe pads, namely those on the third and fourth digits of the fore limbs and hind limbs. Frogs were killed via a lethal dose of Benzocaine. The toe pads were immediately cut from the dead frogs, washed and then fixed in 2.5% glutaraldehyde in phosphate buffer for 24 hr at pH 7.4.

2.1. Scanning and transmission electron microscopy

The glutaraldehyde-fixed samples were rinsed in phosphate-buffered sucrose, post-fixed in buffered 1% osmium tetroxide, and stained in 0.5% aqueous uranyl acetate.

For scanning electron microscopy (SEM), specimens were then dehydrated in an acetone series and critical point-dried. Samples were mounted and gold-coated before viewing with a Philips SEM 500 scanning electron microscope.

For transmission electron microscopy (TEM), specimens were dehydrated in an alcohol (rather than acetone) series. Samples were rinsed twice in propylene oxide to remove the alcohol, embedded in Araldite resin and polymerised at 70°C. Ultra-thin sections (60–70nm) were cut on a Reichert ultramicrotome. These were then mounted on copper grids, stained with uranyl acetate (2% aqueous solution) and lead citrate, and examined using a Philips TEM 301 transmission electron microscope.

2.2. Light microscopic study (Semi-thin sections)

Toluidine blue staining: the glutaraldehyde-fixed toe pads were post-fixed in 1% osmium tetroxide, stained in 0.5% aqueous uranyl acetate, dehydrated using an ethanol series, and embedded in Araldite resin; sections were cut at 0.5 to 1 µm, then stained using 1% Toluidine blue in 1% borax.

Periodic acid/Schiff staining: The semi-thin sections were deresinised in saturated sodium ethoxide for 15-20 minutes [24]. They were then hydrated through two changes of absolute alcohol, 90 %, 70% and finally washed in running water for 10 minutes. Sections were treated in 1% periodic acid for 10 minutes, and then washed in running water for 10 minutes. The sections were transferred to Schiff's reagent for 20-30 minutes then washed in running water for 15-30 minutes. They were stained in Weigert's haematoxylin for 5-10 minutes, then dehydrated, cleared in xylene and mounted in D. P. X.

Toe-pad sections were examined over a range of magnifications using a Wild compound photomicroscope and selected images were recorded using Photo-Shop v. 7 software (Adobe

Systems, San Jose, CA).

3. Results

3.1. Toe-pad ultrastructural feature in adult *Litoria caerulea*

3.1.1. General feature of toe pad (SEM)

Toe pads in *Litoria* are located ventrally on the distal ends of each digit, and are delineated laterally and distally by a circumferential groove. Figure 1 shows a general plan of *Litoria* toe pad.

In addition to the circumferential groove and lateral grooves, toe pads of *Litoria* may additionally possess one or more vertical furrows which subdivide the toe pad surface into medial and lateral areas. These furrows were seen to be better developed in the toe pads of the hind-limbs compared to those of the fore-limbs (Fig. 2 and 3).

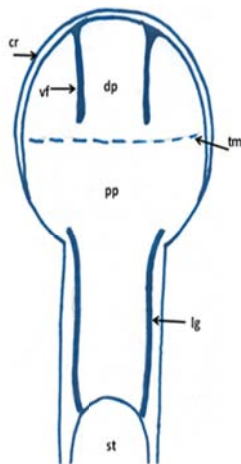


Fig. 1. General organization of tree frog toe pad (ventral view). cg=circumferential groove; dp=distal part; lg=lateral groove; pp=proximal part; tm=transverse margin; st=subarticular tubercles; vf=vertical furrow

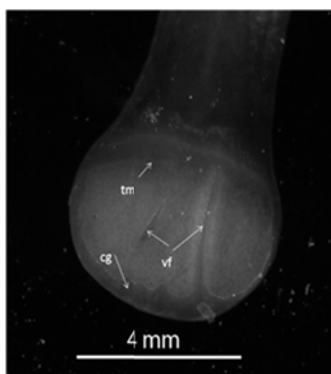


Fig. 2. Toe pad with one large and one small vertical furrow attached to glass plate. Photographed from a live sample, hind limb, cg= circumferential groove; tm= transverse margin; vf=vertical furrow

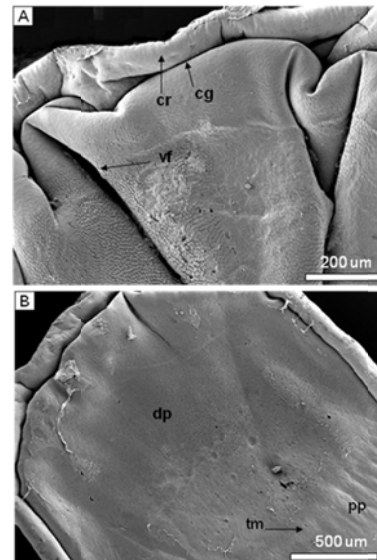


Fig. 3. Scanning electron micrographs of toe pads from ventral view. A: 3rd toe of hind limb pad; two prominent grooves have developed in the hind limb pad, separating each of the pads into a medial and two lateral parts. B: 3rd toe of fore limb pad; lateral grooves in fore limb pad are poorly-developed or absent and the pad surface is smooth and flat. cg=circumferential groove; cr=circumferential ridge; dp=distal part; vf=vertical furrow; pp=proximal part; tm=transverse margin

There is also a transverse margin, separating the distal part of the pad (the adhesion part) from proximal part (non-adhesion part). Subarticular tubercles also exist.

The distal part of the pad is occupied by columnar epidermal epithelia, while the proximal part is occupied by normal epidermal epithelia. Channels (fissures) are visible between columnar cells in the distal area, while there is a tight link between the cells in the proximal area.

3.1.2. Columnar epidermal cells of the toes

Two basic shapes, pentagonal and hexagonal, of epidermal cells are arranged very regularly across the surface of the pad. Very dispersed heptagonal cells are also occupied some regions of the toe. The hexagonal cells are dominant and occupy almost all regions of the toe; while the pentagonal mainly occupies the most distal part of the toe. Most of the hexagonal cells point slightly backwards. The cells are separated by deep intercellular channels. The channels between the columnar cells of the toe pad are highly variable in width. In the most distal part of the toe, the cells are tighter and the widths of the channels reduced (Fig. 4).

A high magnification view of the surface of a single hexagonal/pentagonal cell shows the cell to be covered with nanopillars with somewhat flattened tips, and peg-like projections (Fig. 5).

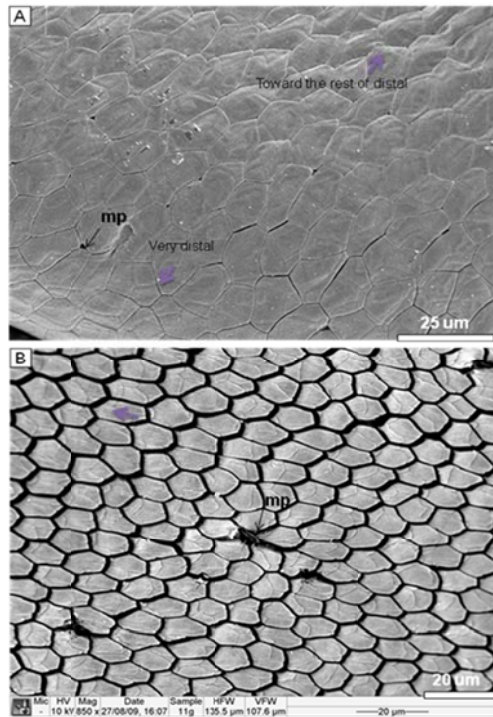


Fig. 4. Scanning electron micrograph of skin surface of toe pad (3rd toe of fore limb). A: from the most distal part of a toe, showing tight epithelial cells. B: from the rest of the distal toe pad, indicating epithelial cells with well-developed channels separating the cells. The arrow in figure B pointing toward proximal. mp=mucous pore

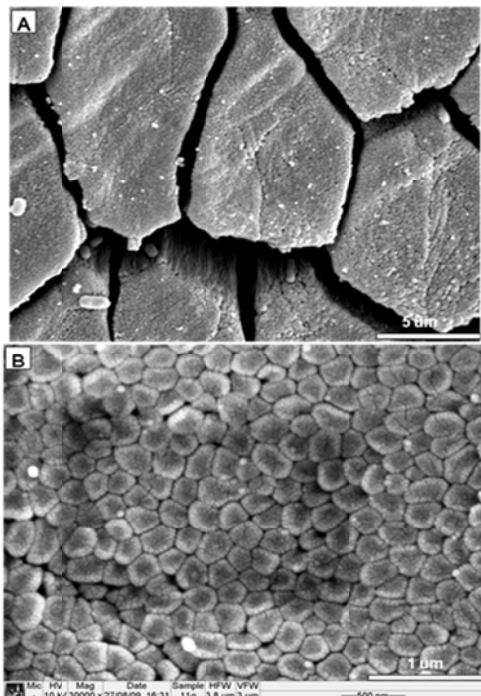


Fig. 5. A: Medium resolution scanning electron micrograph from the toe-pad surface cells (examined from the 3rd toe of fore limb). B: High resolution view of the surface of a single hexagonal/pentagonal cell from the 3rd toe of fore limb, showing that the cell is covered with peg-like projections (nanopillars)

3.1.3. Mucous pores

Three types of mucous-secreting pores are seen between the epithelial cells.

Type I: pores which are simple, without any modification; type II: pores in which the sides of the cell facing the lumen are modified in comparison to the normal striations, and type III: pores which have their own ducts.

The type I pores are more concentrated on the very tip of the distal part of the toe (Fig. 6), and randomly and very dispersedly within the distal part of the toe, while types II and III are distributed in considerable numbers over the entire surface of the toe (Fig. 7). Pore size and shape were variable. Epithelial cells on the circumferential ridge are highly microporated.

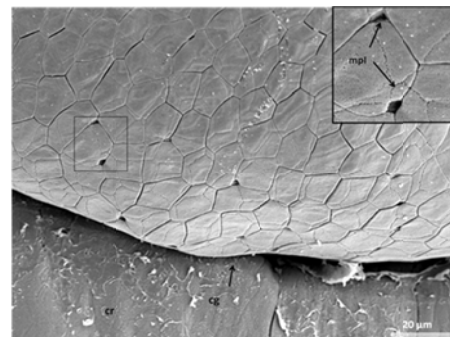


Fig. 6. Low resolution scanning electron micrograph of the very tip of the distal part from a 3rd toe of fore limb, showing type I mucous pores concentrated in this area, cg=circumferential groove; cr=circumferential ridge; mpI=mucous pores

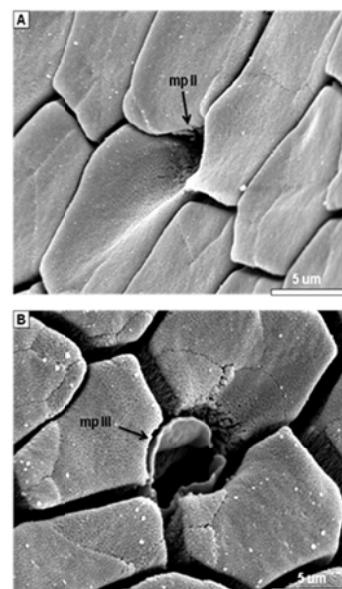


Fig. 7. High resolution scanning electron micrograph from mucous pores (examined from 3rd toe of fore limb). A: type II and B: type III, mp II=mucous pore type II; mp III=mucous pore type III

3.2. Toe-pad histology in *Litoria caerulea* (sections)

Semi-thin sectioning (Fig. 8) shows that the toe pad epidermis in hind limbs consists of four layers. The outermost epithelial cell layer is large but cuboidal in shape and the innermost layer is of columnar epithelial cells. In forelimbs, the epidermis consists of 3 layers.

The outermost epithelial cell layer is of columnar epithelial cells and the innermost layer is of fewer columnar epithelial cells. The outermost layer of both forelimbs and hind limbs is keratinised and non-living, but stains darker in the sections in hind limbs than in the forelimb region, demonstrating denser tonofilament in the hind limb. The nanopillars (the distal part of the epithelial cell) are not located radially to the proximal part of the cell underneath, where they extend from, but are translocated somewhat spirally or obliquely from under the cell layer; therefore, the channels between nonopillar regions is not in the line between the basal parts of the same cells.

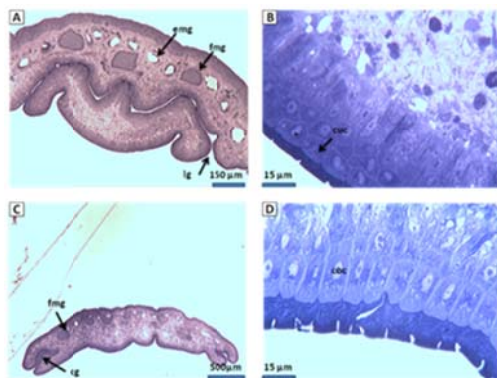


Fig. 8. Semi-thin transverse sections from toe-pad region. A: Low magnification from the 3rd toe of hind limb showing two different shapes of mucous glands close to the upper part of the pad and very deep groove in both sides of the pad. B: High magnification of figure A, showing different layers of epithelial tissue of ventral pad area (note the outermost cell layer which is cuboidal). C: Low magnification from the 3rd toe of forelimb indicating the same structure of hind limb, apart from a lack of or reduction in vertical furrow in the ventral part of toe pad. D: High magnification of figure C, showing different layers of epithelial tissue (note the outermost cell layer, which is columnar), cg=circumferential groove; coc=columnar epithelial cell; cuc=cuboidal epithelial cell; emg=empty mucous gland; fmg=filled mucous gland; vf=vertical furrow

The keratinised layer as an outermost non-cellular layer of skin is seen in all areas of a digit, but it is

shown to be much thicker in the toe-pad region. In addition, there is lack of nonopillar regions and channels in the skin surface areas out of toe pad border. In forelimbs, the skin of the dorsal side of the toe pad consists of 4-5 layers, with flat cells in the outermost epithelial cell layers. In hind limbs, this area consists of 5-6 layers, almost cuboidal (Fig. 9).

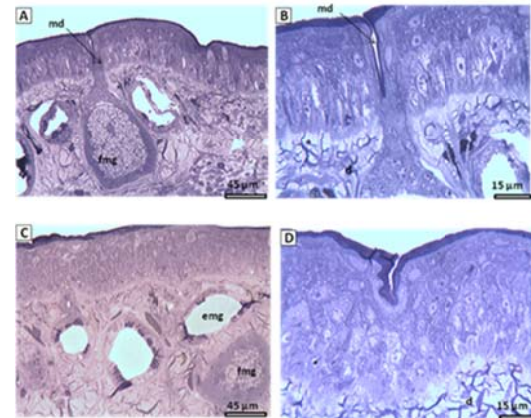


Fig. 9. Semi-thin transverse section from dorsal part of the 3rd toe-pads. A: Low magnification photograph from the epithelium and dermis underneath the forelimb, showing a large vesiculated gland with a clear duct leading to the surface. B: High magnification of A, showing the structure of epithelial tissue. C: Low magnification photograph from the epithelium and dermis underneath the the 3rd toe pad of hind limb, showing some cavitated glands. D: High magnification underneath the hind limb, showing the structure of epithelial tissue, d=dermis; emg=empty mucous gland; fmg=filled mucous gland; md=mucous duct

The underlying dermis is highly vascularised in the ventral part of the toe, blood vessels being identified by the presence of nucleated red blood cells within them. Two types of mucous gland, one filled and another with a large central cavity, are located dorsally, just beneath the dorsal epidermis, and the presence of mucous was confirmed by periodic acid/Schiff staining.

The TEM study clearly shows different stages of gland maturation. The glandular cells of some wide lumen glands are seen to contain either large and often polymorphic secretion granules or relatively large electron transparent vesicles; however, glands full of transparent vesicles are also found. Near the mucous glands, a number of nerve bundles can be found, generally containing several myelinated and un-myelinated nerve fibres (Fig. 10).

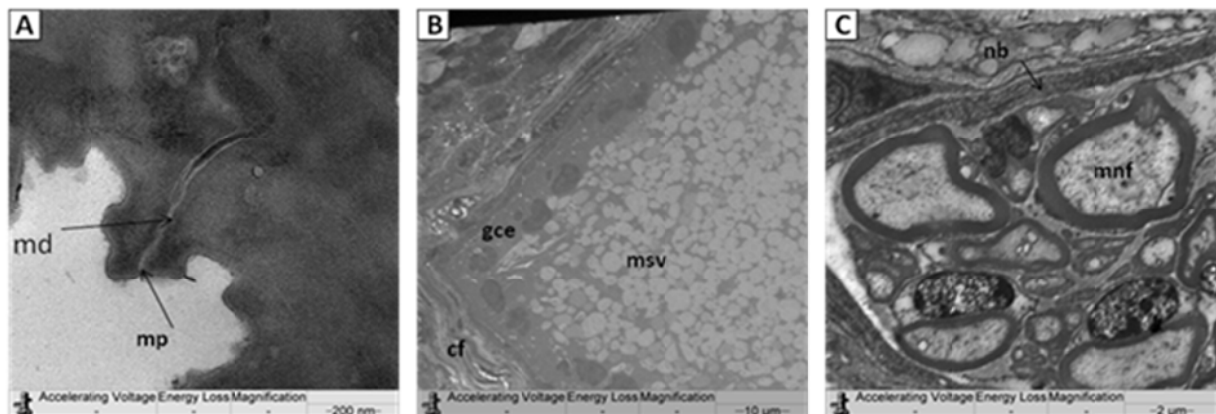


Fig. 10. Transmission electron micrograph of the 3rd toe of hind limb showing A: a gland duct leading to a pore located on the ventral part of the toe-pad; B: mucous gland with mucous secreting vesicles and supporting tissues; C: a nerve bundle found beside a mucous gland. cf=collagen fibres; gce=gland cell epithelium; md= mucous duct; mnf=myelinated nerve fiber; mp=mucous pore; msv=mucous secreting vesicles; nb=nerve bundle

Many of the glands lead to the dorsal surface by clear ducts. In some sections, the distal part of a mucous duct can be seen among the uppermost epithelial cells in the ventral surface. Such ducts will transport mucous to the surface of the toe pad, although there were a very few ventrally-located mucous ducts with no clear link to the mucous glands.

4. Discussion

We present in this article a detailed description of normal adhesive toe-pads in one species of tree frog, *Litoria caerulea*. Our survey of the literature indicates that there is wide range of studies on the toe-pads of different families of frogs [1, 3-8, 10-12]. Although fine differences in structure in the pads of different groups and species are reported, the pad epithelium is nonetheless significantly similar across the arboreal species and even in some non-arboreal species [11]. It is likely that they have developed similar adhesive toe-pads in response to similar environmental pressures, demonstrating morphological and ecological convergence [25]. However, Ohler [11] has observed differences in the shape of toe pad epithelial cells among ranoid frogs adapted to living on wet and flooded rocks, thought to be adaptations to allow rapid removal of excess water from under the pads.

Various mechanisms are used by animals to bond to surface and to avoid falling. Animals can generate forces in three different ways that oppose gravity; (1) by interlocking the surface of the animal with that of the support, e.g. by use of claws; (2) by friction, that involves both micro interlocking and intermolecular forces between materials at points of contact, e.g. primates; (3) by bonding, between the animal and its support. Functionally, these bonding mechanisms can be

separated into dry adhesion (geckos and spiders adhering through van der Waals forces) and wet adhesion (tree frogs and insects adhering by capillarity). Structurally, they can be divided into hairy adhesive pads (geckos and insects such as flies and beetles) and smooth adhesive pads (such as tree frogs and insects such as stick insects and grass hoppers).

Scherge and Gorb [26] and Persson [27] indicate that the similarity between the adhesive pads of tree frogs and grasshoppers is remarkable, indicating highly optimised (by natural selection) systems for adhesion. However, many smooth adhesive pads in insects do not utilize a hexagonal pattern, and although many climbing animals use claws, others use adhesion. A few animals also use suction, including disk-winged bats and clingfish.

4.1. General morphology

Hertwig and Sinsch [3] suggested that the digital specialisation varies within the same individual between each finger and toe. They indicated that the expansion of the pad and also the form of the circumferential groove and ridge are related to the relative contribution of each digit to climbing. Therefore, they argued that interspecific comparisons of toe morphology are only reliable if they refer to the same toe as having the same function in the compared species. In our literature, there are many comparative works but very few reports have noticed this point and considered that there is inter-digital variation in the same forelimb or hind limb of an individual.

Furthermore, there is a lack of data showing any fine, different structure of toe-pads between forelimb and hind limb in the same individual. Ba Omar et al. [28] in their studies on the development of adhesive toe-pads in *Phyllomedusa*, investigated

the toe-pads in both forelimbs and hind limbs, and although they have reported details of toe-pad development for both locations, there was no comparative investigation. They concluded that development in forelimbs was slightly more advanced than that of hind limbs.

In the toe pads of *Litoria caerulea* I investigated the differences between hind limbs and fore limbs and found the vertical furrow were more developed in hind limbs than forelimbs (Fig. 3).

Scholz et al. [20] also reported the circumferential groove running around the top and sides of each toe pad. Although they mentioned that the two prominent grooves develop which separate each of the pads into a medial and two lateral parts, which limbs this structure (vertical furrow) is found on is unclear. Another study by Wüttke, et al. [29] on the grooves of toe pad of about 16 *Litoria* frogs of different ages showed that some of them had no vertical furrow but in others the number of grooves varied from 1 to 5 and was correlated with frog size.

Lee, et al. [9] compared the toe pads of 11 species of Rhacophoridae and Hylidae tree frogs. The results indicated that the same pattern of circumferential groove and transverse groove on the toe pads was found among all members of *Rhacophorus*, but the lateral groove was absent. The study on hyla species (*Hyla chinensis*) by Lee et al. [9] indicated that the transverse furrow and lateral groove are absent and the circumferential groove did not extend to and around the proximal margin. Of interest was the presence of a short vertical furrow on the hind limb, which was not seen in *Rhacophorus*.

4.2. Epithelial layer

Figure 3 showed the most hexagonal epidermal cells occupying the surface of the pad in the *Litoria* species, elongated proximo-distally which pointed slightly backwards. Among the various ranoid frogs studied by Ohler [11], some species had epidermal cell surface with regular outline on the digital pad, but most of the ranid species had no regular outline but were elongated proximo-distally. Ohler [11] showed on the narrow distal side, the elongated cells have more or less developed projections; specifically there were well developed projections in the genus *Amolops*.

Typically, the toe at the ventral side consists of six to eight cell layers in *Hyla cinerea* [2], which differentiate gradually into columnar cells at the top. The cytological analysis of the toe pad by Hertwig & Sinsch [3] in marsupial frogs were also comprised of six cell layers.

The innermost four layers of epidermal cells of the toe pad did not differ in structure from those of

normal skin, but in the fifth layer, the shape of cells changed from cubical to prismatic.

However, Ba Omar [28] found the epithelial layer in *Phyllomedusa trinitatis* to have 12 or so layers in adult pads, which seems to be an exception among toe-pad epithelial thicknesses.

Our finding in *Litoria caerulea* is that the number and the cell shape of the toe-pad epidermis layer in hind limbs are different from those of fore limbs. The hind limb consists of four layers. The outermost epithelial cell layer is cuboidal in shape and the innermost layer is of columnar epithelial cells. In contrast, in forelimbs the epidermis consists of three layers. The outermost epithelial cell layer is columnar and the innermost layer is of less columnar epithelial cells.

Further, we cannot exclude the possibility that this is related to pad size, i.e. smaller pads having fewer layers, or it is perhaps dependent to the stage of development.

Although these variations exist between different species as well as between the forelimbs and hind limbs of any individual, there is no known functional relationship for this feature either.

Green [5] suggested that if digital characters are useful in the classification of the frogs, the accuracy of such data would be enhanced and more characters could be observed through the further use of the scanning electron microscope. Green [5] argued that the size of the pad cells could thus be used to distinguish between the two species with high probability. My view is that even the outermost columnar epithelial cells change at their apices into peg-like projections with different shapes (pentagonal, hexagonal or heptagonal tips) and the distribution of these columnar cells with differently-shaped tips can be computerised and analysed, taking into consideration many other surface characteristics. Hence, a way may be found not only to distinguish the two species, but also for potentially distinguishing individuals in a species by fingertip examination. We may need to take this in to consideration if there are minor changes in the detailed pattern of cell shape every time the outer cell layer is sloughed off at a molt.

4.3. Mucous pores and mucous glands

Green [5] showed that there is remarkable variation in the numbers of mucous pores associated with the toe-pads of various species. Lee [9] reported no mucous visible on the outermost epidermis of any tree frog he examined. Ohler [11] argued that the prismatic cells, the channels and the mucous glands are required in the humidification mechanism necessary for sticking. On the other hand, Nachtigall [30] showed that even distilled water has sufficient adhesive ability as an adequate

intermediary fluid to affect adhesion. Thus, he concluded that mucous, or even particularly sticky mucous, need not be mandatory for the functioning of the pads. Ernst [22] believed that the mucous glands present in the dermis of the digital pads are not fundamentally different from similar glands found elsewhere in the skin of tree frogs. We found three types of mucous pore (type I, II and III) dispersed on the surface of toe pads, but the specific function for each type of pore has not been investigated so far. Although mucous pore type III is visible in some published micrographs, for example, Rivero, et al. [31], Figs. 7, 8, 10, in the toe-pad of species *Eleutherodactylus coqui*, this feature has not been noted before. We also noticed a significant number of mucous glands on the dermis of the digital pads, and it is somewhat interesting that during the serial sectioning of the toe pad, many of these glands were found to have their ducts pointing to the dorsal surface rather than to the ventral surface of the digital pads. For the rest of the glands, no clear link between the glands and the ducts was seen. The very close location of the glands in the dermis to the dorsal surface of the toe pad suggests that the rest of the glands may also be related to the upper part of the pad. Noble and Jaekle [1] reported that these glands originate in the central or upper part of the pad, continuing ventrally into shorter and narrower ducts which perforate the epidermis at an acute angle. Our PAS staining showed some mucous-secreting cells and micro ducts on the ventral surface of the toe pad, which can support the source of mucous secretion to the ventral surface of the toe pad, but we did not find any link to the main glands in the dermis.

I found a dense network of capillaries and lymph sacs in the dermis beneath the toe pad. These sinuses are believed to play an important role as shock absorbers during landing after a jump [19]. These structures produce a hydrostatic pressure for a safe landing, but may also make the toe pad sufficiently flexible to transform the pad to a suction form for better adhesion when the blood drains away to the main circulatory system by pressing the toe pad to the surface.

SEM and semi-thin sections indicate that the hind limb digital toe-pad is deeply grooved in lateral parts as a marker to diagnose hind limb from forelimb digital pads, but it also increases the surface of the digital pad in hind limbs, probably for stronger adhesion. Having such grooves allows larger pads to conform better to surface irregularities.

Hydrostatic pressure induced by the sinus could also help to expand these, folding when necessary. Apart from this suggested role, the grooves being the same as other characters on the toe pad could be considered as species recognition.

5. Conclusion

Fine differences in structure exist between the pads of forelimbs and hind limbs in *Litoria caerulea* species in the extent and existence of the circumferential groove, and in the organisation of the surface-cell epithelium.

The number, type and distribution of mucous pores, and the number of epithelial layer are also different from those of other hyloid species. Together, all these studies indicate that anuran adhesive pads are a remarkable example of convergent evolution, but some prominent characteristics of the toe pads still need to be clarified in terms of the relation between their forms and their functions. In general, apart from different structure and function of anuran adhesive pad, there is various adhesive systems and mechanisms among the animal kingdom, some examples have been given in the discussion part.

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