Comparison of toe pads of some southern African climbing frogs

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The morphology of the toe pads of eight species of *Hyperolius* (Hyperoliidae), *Chiromantis xerampelina, Leptopelis natalensis* and *Kassina maculata* (Rhacophoridae) was examined using a scanning electron microscope. Disc width, margin width and toe pad cell size are variable in *Hyperolius*, but not directly related to frog size. The rhacophorids were found to have type II mucous pores, contradicting earlier findings. SEM work on toe pads is not taxonomically useful at the species level. We suggest that the circumferal groove may play a role in retaining liquid essential for the adhesion of tree-frog toe pads to smooth surfaces.

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Die morfologie van die toonkussinkies van agt spesies van *Hyperolius* (Hyperoliidae), *Chiromantis xerampelina, Leptopelis natalensis* en *Kassina maculata* (Rhacophoridae) is met behulp van 'n skandeer elektronmikroskoop ondersoek. Skyfiebreedte, randbreedte en toonkussinkie-selgrootte varieer in *Hyperolius*, maar hou nie verband met die grootte van die padda nie. Die Rhacophoridae het tipe II slymgaatjies, wat 'n weerspreking is van vorige werk. SEM-werk op toonkussinkies het geen taksonomiese waarde op die spesiesvlak nie. Ons stel voor dat die omtreksgroef 'n rol speel by die berging van vloeistof, wat noodsaaklik is vir die vaskleef van boompadda-toonkussinkies aan gladde oppervlaktes.

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Climbing frogs possess expanded adhesive pads on the tips of their digits. These terminal discs serve to increase the surface area of the toes to improve the frog's grip. Larger tree-frogs make use of additional friction areas in the form of subarticular tubercles.

This study was initiated to investigate the taxonomic usefulness of toe discs, especially in the genus *Hyperolius* where reliable identification of some preserved material is very difficult. We also attempted to complement a scanning electron microscope study by Green (1979) of the toe pads of tree-frogs mainly from the Americas.

Southern African genera adapted for climbing by possessing toe discs include *Hyperolius, Chiromantis, Leptopelis, Kassina, Heleophryne, Phrynomerus, Natalobatrachus* and *Afrixalus*. This report is based on specimens of the following species (sample size in parentheses): *Chiromantis xerampelina* (1), *Leptopelis natalensis* (1), *Kassina maculata* (1), *Hyperolius marmoratus* (9), *H. pusillus* (2), *H. nasutus* (1), *H. argus* (1), *H. semidiscus* (1), *H. horstocki* (1), *H. tuberilinguis* (3), *H. pickersgilli* (1). Green (1980) demonstrated that the cells of toe discs show little variation from one specimen to another in the same species.

The vegetation upon which these species climb is broadly similar (reeds, grass and other aquatic vegetation) although *Leptopelis natalensis* is commonly found in trees.

Methods

We removed the second toe from the left forelimb of freshly killed specimens. These were fixed and stored in glutaraldehyde. Standard SEM preparation was used: specimens were soaked in 2,5% glutaraldehyde in 0,2M sodium cacodylate buffer, rinsed twice in the same buffer, refixed in 2% osmium tetroxide in 0,2% sodium cacodylate, rinsed twice in buffer and then dehydrated through a series of ethanols and brought to acetone. The specimens were then prepared in a critical point dryer and sputter coated with gold. Fresh specimens were examined using a Cambridge 180 Stereoscan.

Results

Disc morphology

The discs show a range of variation in respect of size and the presence of a circumferal groove. Within *Hyperolius*, disc width varied between 0,6 and 1,4 mm, but is only weakly related to frog size (Figure 1). Some toe pads occupy the whole disc, while others are surrounded by a margin. Like the discs, margin width is not related to frog size. Specimens of *H. nasutus*, *H. horstocki* and *H. pickersgilli* were emarginate. However, the larger tree-frog genera (*Chiromantis*, *Leptopelis* and *Kassina*) do have wider margins and proportionally larger discs. A deep circumferal groove separated the pads from the margins in the specimens examined (Figure 2).

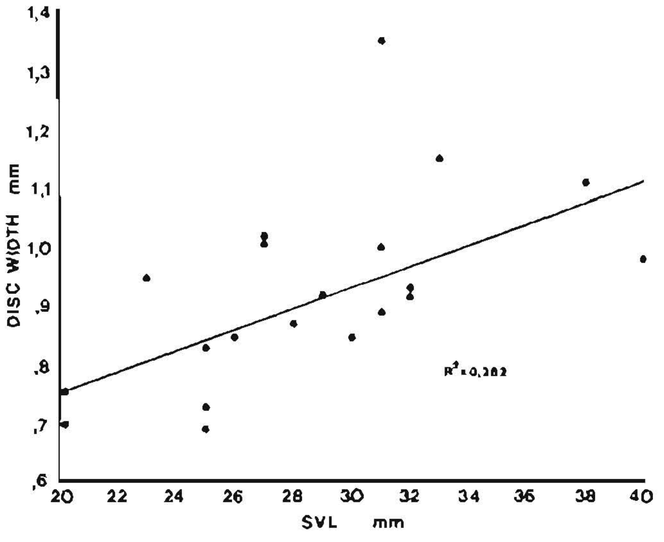

Figure 1 The relationship of toe disc width and snout-vent length in *Hyperolius*.

Figure 2 (A) Emarginate *Hyperolius nasutus* toe (Dukuduku). Scale = 100 μm. (B) Toe of *H. semidiscus* showing margin and groove (Howick). Scale = 166 μm.

Toe pad cells

The toe pads are covered with living polygonal columnar cells. The details of cell structure in the specimens examined in this study are remarkably similar to those illustrated and described by Green (1979). The cells are modified by being elongated from the base and having separate apices. Frogs rely mainly on surface tension for adhesion (Green 1981) and the spaces between the cells (Figure 3) provide a suitable rough surface for the wetting agent, consisting of the cell coat, mucus and surface water (Green 1981). Individual cells are able to find the best angle for adherence to the surface irregularities. As such a surface tension bond is most resistant to a pull at right angles to the substrate, the cells are reinforced by a basal-apical cytoskeleton of tonofilaments (Green 1979). These tonofilaments strengthen the cell to resist the vertical pull associated with surface tension adhesion (Green 1981). The reinforcing cytoskeleton is represented by filamentous structures visible on the sides of the cells as strands running from base to apex (Figure 4).

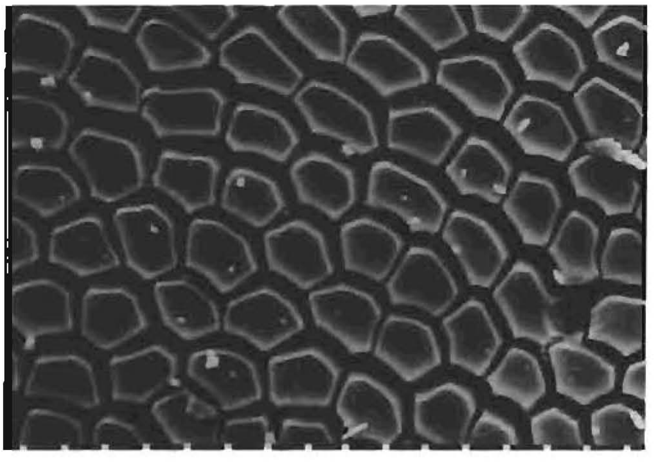

Figure 3 Columnar toe pad cells of *H. nasutus* (Dukuduku) showing spaces between the cells. A type 1 pore is visible lower right. Scale = 10 μm.

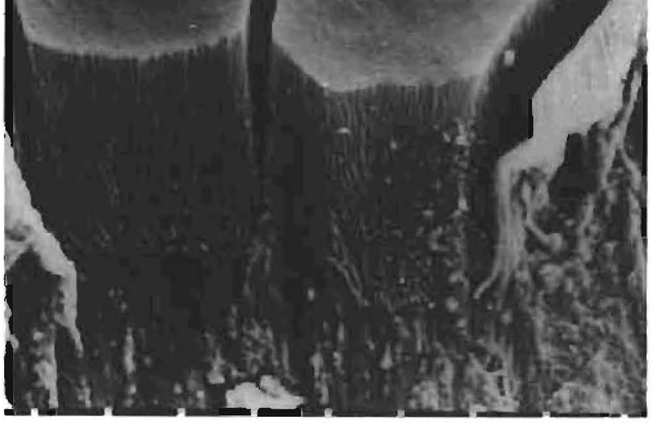

Figure 4 Sides of toe pad cells of *H. tuberilinguis* (Gonubie) showing the filamentous structures. (See text.) Scale = 3μm.

Mean surface areas of toe pad cells were calculated from SEM photographs. The mean areas of the cell apices varied from 66 μm^2 to 172 μm^2 (Table 1) being smallest in the genera with large toe pads (*Kassina* and *Leptopelis*). Cell area is, however, extremely variable, *Hyperolius marmoratus* being 105 μm^2 to 155 μm^2.

Table 1 Mean apical surface area of the toe pad cells, number of mucous pores per μm^2 and the number of cells used to calculate surface area

Species	Locality	Mean surface area (μm^2)	Pores per mm^2	Number of cells
Leptopelis natalensis	Port St. Johns	89	17	20
Kassina maculata	Dukuduku	66	75	11
Chiromantis xerampelina	Mkuze	135	175	10
Hyperolius marmoratus	Port St. Johns	121	33	10
	Howick	155	FEW	10
	Gonubie	105	100	15
	Gonubie	116	FEW	10
	Port Shepstone	109	22	10
	Jeffrey's Bay	127	125	11
	St. Lucia	123	33	10
H. pusillus	Port St. Johns	154	50	13
	St. Lucia	118	125	14
H. argus	Dukuduku	167	100	13
H. horstocki	Stanford	172	325	10
H. tuberilinguis	Port Shepstone	138	83	11
	St. Lucia	133	22	11
	Port Shepstone	161	125	16
H. semidiscus	Port Elizabeth	117	FEW	12
	Howick	130	75	11
H. nasutus	Dukuduku	163	33	10
H. pickersgilli	Avoca	150	22	10

Mucous pores

Mucous pores are visible between the cells on some toe pads (Figures 5, 6). Ernst (1973) classified these into two types on the basis of the sculpturing of the cell wall facing the pore. Type I pores are simple, with unmodified walls resembling the striations occurring on the sides of the cells not facing pores. Type II pores are modified, the cell walls showing sculpturing in the form of rough finishes or funnel-shaped openings to the pores. *Hyperolius* has type I pores, while the other three genera all possess type II pores.

The presence of pores on the toe pads is very variable. Some pads do not have any visible pores, while others may have large numbers of pores. *H. marmoratus* from Gonubie displayed very few pores in one individual, and about 100/mm^2 in another individual. Relative pore densities are listed in Table 1.

Marginal-groove epithelium

Although the surfaces of the toe pad cells were virtually identical in all the specimens examined, the surface details of the epithelium of the margin surrounding the toe pads were generically distinct. *Chiromantis* and *Hyperolius* share a pattern of spherical knobs with a central depression in each, while *Kassina* possesses raised vermiform structures. *Leptopelis* has a spongy, perforated cell surface (Figure 7). *Hyla chrysocelis* also appears to have this spongy epithelium in the illustrations in Green (1979). Type II pores are clearly visible in the marginal epithelium of *Kassina*.

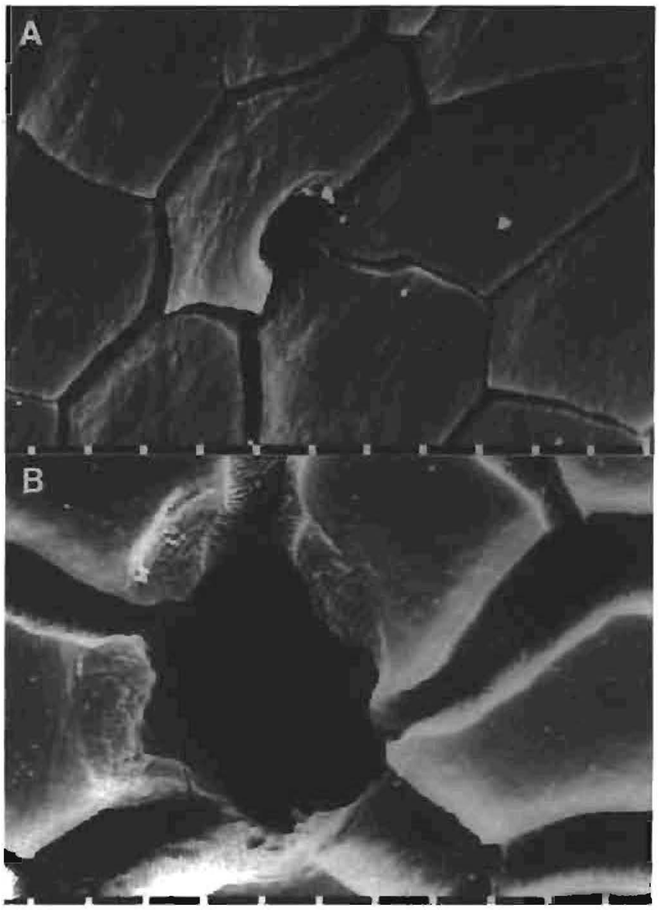

Figure 5 Type I mucous pores: (A) *H. semidiscus* (Port Elizabeth). (B) *H. nasutus* (Dukuduku). Scales = 3μm.

Discussion

Our results indicate that scanning electron microscopy of tree-frog toe pads has little value as a taxonomic tool at the species level, but does provide information for use in higher categories. The African species examined here were microscopically very similar to the mainly American species described by Green (1979).

Green (1979) noted a similarity in number and type of pore in *Polypedates leucomystax* and *Hyperolius viridiflavus*, using this as supporting evidence of the common ancestry of the Rhacophoridae and Hyperoliidae as proposed by Liem (1970). We confirm that *Hyperolius* has type I pores, but find that *Chiromantis*, *Kassina* and *Leptopelis* have type II pores. As these large tree-frogs are placed in the family Rhacophoridae (Schiøtz 1967) this contradicts Green's suggestion above. The affinities of these four genera are further complicated by studies of the marginal epithelia. *Chiromantis* shares a common pattern with *Hyperolius*, but *Kassina* and *Leptopelis* each have different patterns. The similarity of the functional anatomy of the toe pads reflects a common function, and does not appear to be taxonomically useful.

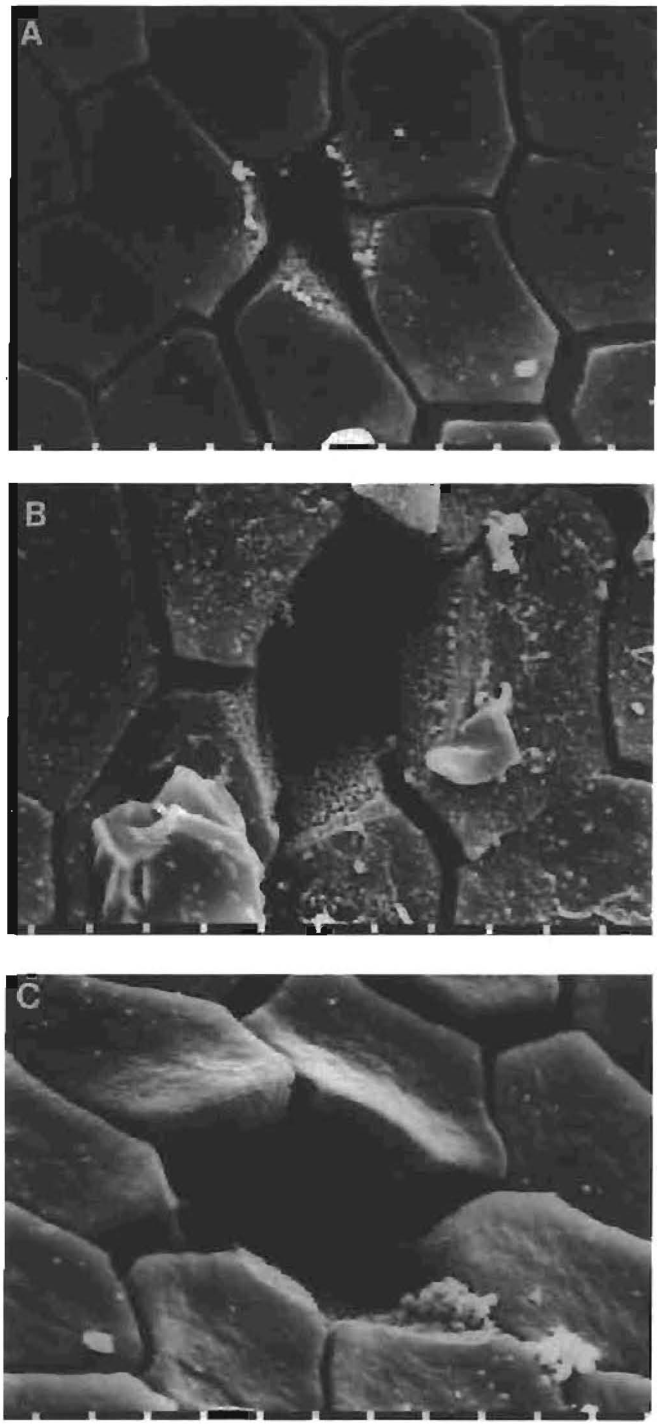

Figure 6 Type II mucous pores: (A) *Kassina maculata*, (B) *Chiromantis xerampelina*, (C) *Leptopelis natalensis*. Scales = 3μm.

Although Green (1979) believes that the circumferential groove surrounding the toe pad is present only because of two discontinuous tissue types — squamous epithelium and columnar pad cells — and mentions no role for this groove in toe function (Green 1981), we suggest that the groove may serve as a reservoir for the fluid wetting agent in those species where it is well developed. Frogs which move from wet to dry substrates may take along a little moisture in each groove, which could be expressed by pressure on the disc, ensuring that there is sufficient liquid for the surface tension bond to be effective.

The variation in cell areas in *Hyperolius marmoratus* indicates that some specimens have toe pad cells with

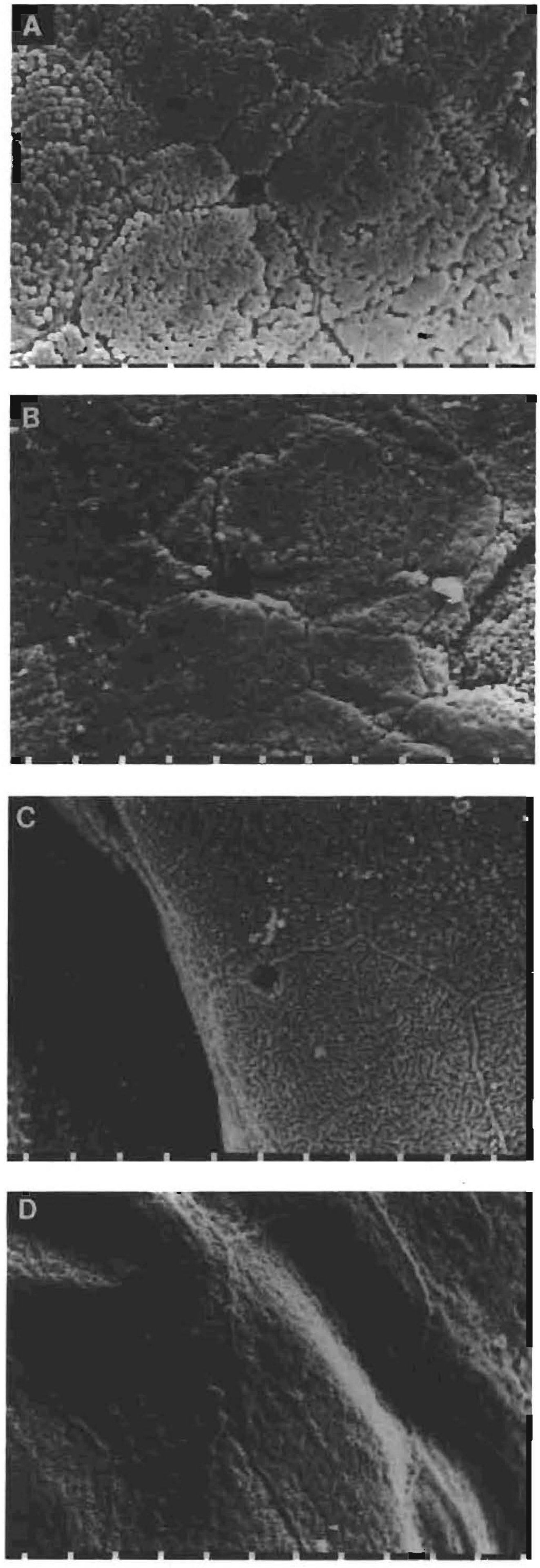

Figure 7 Surfaces of marginal epithelia: (A) *Hyperolius marmoratus* (Port Shepstone), (B) *Chiromantis xerampelina*, (C) *Kassina maculata*, (D) *Leptopelis natalensis*. Scales = 3μm.

estimated cell volumes about 1,9 times as large as others (1929 μm^3 Howick; 1075 μm^3 Gonubie). These represent the extremes, with a mean estimated cell volume for *H. marmoratus* of 1359 μm^3. Although the 1,9 times difference in cell volume is remarkably close to the 1,8 times difference found by Green (1980) between diploid and tetraploid *Hyla versicolor* we found no evidence of polyploidy in a small sample of *Hyperolius*. However, the genus is highly polymorphic for colour, and it may be that this polymorphism is reflected in the size of toe pad cells.

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