Passive Diffusivity of Water and Sodium Through the *Stratum Corneum* of Three Species of Aquatic Snakes

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Master of Arts

by

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1991

APPROVAL SHEET

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the requirements of the degree of

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Dedication

To my parents,

who have always encouraged me.

And where the water had dripped from the tap in a small clearness,
He sipped with his straight mouth,
Softly drank through his straight gums into his slack long body,
Silently.

"Snake"
D. H. Lawrence

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ACKNOWLEDGMENTS

I thank my friend and major professor, Dr. John Musick, for his support and encouragement during the course of this research. I am also very grateful to all the members of my committee, G.R. Brooks, Robert George, William MacIntyre, and Charlotte Mangum, for helpful suggestions regarding this study and for thoughtful review of the manuscript.

Thanks are due to many members of the VIMS community whose help was instrumental in making this study possible. Susi Sami kindly helped with German translations. Nita Walker willingly and jovially prepared the histological specimens. Patrice Mason was indispensable during the course of the electron microscopy. Dave Zwerner, Beth McGovern and Wolfgang Vogelbein gave freely of their time during histological examinations. Jiangang Luo aided freely in computer graphics.

The library staff, including Diane Walker, Susan Barrick, Janice Meadows, and Marilyn Lewis contributed much time and energy on countless occasions; without their kindness this study would have been impossible. Very special thanks are due Kay Stubblefield and Bill Jenkins, for countless reasons.

Deep gratitude is extended to Ray Floriano, Virginia Commonwealth University, for helping to redesign the glassware used in the diffusion experiments, and in finally constructing it.

The most special thanks of all is extended to Ernie Warinner, whose kindness, patience, and understanding during difficult and dangerous times will never be forgotten.

Bonnie J. Ambrose typed the manuscript.

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ABSTRACT

Shed skins of two species of acrochordid snakes and one homolopsinid snake were examined in order to determine the permeabilities of water and sodium. Permeabilities for *Acrochordus granulatus* revealed higher uptake of water and low sodium influx, a situation typical of marine snakes. Permeabilities of *Acrochordus javanicus* more closely resembled estuarine species in that *A. javanicus* remains iso-osmotic to its medium. Shed skins of *Erpeton tentaculatum* displayed permeabilities with greater uptake and retention of sodium, a characteristic of freshwater snakes.

Integumental histology was studied in *A. granulatus* and *A. javanicus*. Both species displayed features typical of the skin of all lepidosaurs. Scanning electron microscopy revealed scale topographies unique to acrochordates, although *A. granulatus* and *A. javanicus* displayed interspecific variation. The microdermatoglyphics of *E. tentaculatum* resembled the topography found in the homolopsinid genus *Cerberus*.

Microdermatoglyphic structure reflects adaptations to specific ecological conditions in the genus *Acrochordus*.

INTRODUCTION

All marine, freshwater, and land reptiles maintain the same extracellular body fluid concentration of electrolytes (Dunson, 1979). However relatively few reptiles have been successful in colonization of the marine environment because of their inability to maintain a hypo-osmotic condition of body fluids with respect to the ambient medium (Dunson, 1984). An evolutionary trend among the reptiles is the development of extrarenal or extracloacal mechanisms to cope with the excretion of excess electrolytes, regardless of the state of hydration. This is a consequence of the inability of the reptilian kidney to secrete urine hyperosmotic to the body fluids (Dantzler, 1976). Since Schmidt-Nielsen and Fange (1958) discovered lacrymal and lingual salt glands in reptiles, much attention has been given to the role of these organs in osmoregulation in reptiles. Although the importance of salt glands in osmoregulation should not be underestimated, they are only a portion of a more integrated system that regulates both the intake and output of salts and water (Dunson, 1984). The initial indication that organs other than salt glands are critical in osmoregulation in marine reptiles stemmed from studies recognizing a variation in the excretion rate and gland size among salt glands in the Hydrophiidae (Dunson and Dunson, 1974).

An unusual property of serpent skins, asymmetrical diffusion, was first recognized by Stokes and Dunson (1982) and was later extended to marine forms, the Hydrophiidae, by Dunson and Stokes (1983). The most notable aspect of polarization of water and inorganic ion fluxes is that the fluxes are symmetrical in opposing directions, thereby facilitating osmoregulatory balance (Dunson, 1984). Water and inorganic ions diffuse more rapidly across the skin in one direction than in the other when the concentration gradient is the same. In freshwater species water efflux exceeds influx, and inorganic ion influx exceeds efflux. There appears to be a link between the needs of a freshwater snake (to excrete water and save electrolytes) and the direction of the asymmetrical diffusion (higher water efflux and ion influx). The opposite is the case in most marine snakes (higher influx than efflux of water and higher efflux than influx of sodium ions). Therefore asymmetrical diffusion may indeed have an adaptive value for aquatic snakes (Ljungman and Dunson, 1983).

Roberts and Lillywhite (1980) demonstrated the importance of lipids in the permeability barrier of reptilian skin, and suggested that keratin and scale morphology were of nominal importance in limiting ionic exchanges. Subsequently, Stokes and Dunson (1982) proposed a model of channel structure in which cylindrical channels penetrating the protein or protein-lipid membrane are lined with multiple layers of lipid molecules. The depth of the lipid lining and the overall number of channels are envisaged as a characteristic of a given species depending on its specific environmental demands. It is possible that asymmetric diffusion may be related to differences in hydration state of the hydrophobic and hydrophilic ends of lipid molecules that are oriented in opposite directions along each end of the channel. However, the problem remains that ionic channels may well achieve asymmetric diffusion by mechanisms different from that of water channels, since electrolytes are virtually impermeable. Maderson (1988) proposed that ions require a hydrophilic environment for movement through lipid bilayers. Therefore permeation by ions might require integral membrane proteins in the form of channels or carriers.

Few investigators have concerned themselves with diffusion of sodium and water in the aquatic snake family Acrochordidae and in the opistoglyph *Erpeton tentaculatum* (Homolopsinae), likewise an aquatic species. Dunson (1978) has investigated sodium and water fluxes in *Acrochordus granulatus*, but not other acrochordids. Literature is scant on the genus *Acrochordus* as a whole.

Of special interest from the standpoint of osmoregulation are the habitats and ranges given for the three species. *Acrochordus javanicus* Hornstedt, inhabits oligohaline estuaries ranging from Thailand southward through the Greater Sundas; *A. granulatus* Gray, inhabits mangrove swamps and coral reefs in the New Guinea-Solomons region -- both northern and southern coasts of New Guinea, the Bismarck Archipelago, and the Solomon Islands; *A. arafurae* McDowell, is confined to freshwater rivers of Australia and New Guinea draining into the Arafura Sea and westernmost Coral Sea. Since the three species of *Acrochordus* range from freshwater rivers to coastal seas, the mechanisms of osmoregulation may vary according to the varying salinities, tidal cycles, and precipitation regimes encountered throughout the year.

Though not closely allied, *Erpeton tentaculatum* Lecepede, a species sympatric with the acrochordids of Southeast Asia, might be expected to react similarly. Pertinent information on salt and water balance in this species does not exist.

In view of the scant information available on these serpents, this study was undertaken with three objectives: to determine the amount of influx and efflux of water and sodium through the *stratum corneum* of *Acrochordus granulatus*, *A. javanicus*, and *Erpeton tentaculatum*, to describe the basic histology of the skin of *A. granulatus* and *A. javanicus*, and to examine with SEM the microdermatoglyphics of *A. granulatus*, *A. javanicus*, and *E. tentaculatum* to locate pores in the scales through which water and ion passage may occur.

MATERIALS AND METHODS

Live specimens of *Acrochordus javanicus* and *A. granulatus* were obtained through commercial dealers. Both species were maintained in commercial plastic food containers with water temperature *ca* 28-29^{o}C. *Acrochordus javanicus* was maintained at salinity of 5^{o}/oo and pH 6.5-7.0. *Acrochordus granulatus* were maintained at a higher salinity of about 10^{o}/oo and pH 7.0-7.5. All snakes were given acrylic yarn as cover. Snakes were fed twice weekly on goldfish.

Live specimens of *Erpeton tentaculatum* were sent (*gratis*) from the New York Zoological Society. All tentacled snakes were maintained in a 55 gallon aquarium with water temperature *ca* 29^{o}C, pH 6.5, and salinity 2^{o}/oo. Branches were provided for anchorage. *Erpeton* were fed goldfish *ad libitum*.

Shed skins were washed in deionized water, split ventrally, and allowed to air dry pending experimental investigation. In order to remove any attached debris, all skins were cleaned by ultrasonication in deionized water for 10 minutes prior to testing.

WATER FLUX

The permeability of middorsal areas of shed skin to tritiated water was studied at 20-23^{o}C in a glass chamber modified from that of Dunson (1978). One ml tritiated water (10μCi/ml) was placed in the top of the chamber and 16.0, 16.5, and 17.5 ml of 1M NaCl was placed in the bottom each of three experimental chambers respectively. Influx was measured by positioning the outside of the skin next to the isotopic solution. For determination of efflux, the orientation was reversed. Middorsal sections of skin were used alternately for influx and efflux, each section being used only once. The skin was sealed between the two chambers of the testing unit using an o-ring (1.2 cm diam.). Passage through the skin was measured by sampling 1 ml from the lower chamber after a period of one hour. Leak tests using parafilm and latex revealed no passage of isotopes. Samples were mixed with 10 ml Dupont Aquasol-2 and counted on a Beckman LS-5000 TD liquid scintillation counter.

SODIUM FLUX

Sodium flux experiments using $^{22}NaCl$ followed the same routine as water flux. One ml samples were taken at 2 hour intervals and counted in the same manner as water flux samples.

All experimental procedures closely followed those of Dunson and Stokes (1983), and all flux calculations were made according to the following equation, which is a modification of that used by Stokes and Dunson (1982):

$$\underset{1 \rightarrow 2}{\text{flux}} = K_1 W_1$$

$$K_1 = \lambda / 1 + (W_1/W_2)$$

$$\lambda = \frac{2.3}{(t)\ (V)} \log_{10} \left(\frac{W_2}{W_1 + W_2} \Big/ \frac{C_{1_t}}{C_{1_0}} - \frac{W_1}{W_1 + W_2} \right),$$

Where flux (K_1W_1) is in micromoles per ml hour; K_1 is a rate constant; V is the volume of exposed shed skin in ml; W_1 is the water on the initially labelled side of micromoles; W_2 is the water on the side initially unlabelled in micromoles; t is time in hours; $^{C}1_t$ is the total counts per minute in chamber 1 at time t; and $^{C}1_0$ is the total counts per minute in chamber 1 at time 0. Flux values are given in μ mol/cm^2 area · cm length · hour.

HISTOLOGICAL EXAMINATIONS

Histological investigations were undertaken in order to determine the thickness of the shed skin, and to examine the entire integument of Acrochordidae. Pieces of tissue were preserved in Bouin's solution, embedded in paraffin, sectioned at 6 μ and stained with hematoxylin and eosin. In order to better discern the muscle and connective tissues, histological samples were prepared using Masson's trichrome method (Luna, 1968).

MICRODERMATOGLYPHICS

Dorsal scale samples from shed skins of all species were prepared for scanning electron microscopy by mounting on an aluminum specimen mount with scotch double-stick tape. Skins were then coated in a DV-502 vacuum evaporator using 8 inches of gold/palladium wire (60% gold, 40% palladium). All specimens were examined on an Amray 100 scanning electron microscope at different magnifications, as different species showed optimal resolution of features at various magnifications.

DATA ANALYSIS

For determination of statistical significance of ion fluxes, the Mann-Whitney signed rank test was used with α level of .05. (Zar, 1984).

RESULTS

Fluxes

The fluxes of water exceeded the fluxes of sodium chloride for all snake species examined (Table 1; Appendix; Figures. 1 and 2). The influx of water was significantly greater ($P < .05$) in *A. granulatus* than *E. tentaculatum*, and greater in *A. javanicus* than *E. tentaculatum* (Table 2). the efflux of water was significantly greater ($P < .05$) in *A. javanicus* than *A. granulatus*, and greater for *A. granulatus* than *E. tentaculatum* (Table 2). Influx and efflux rates of water were not significantly different ($P > .05$) in each species.

Sodium chloride influxes were significantly greater ($P < .05$) in *E. tentaculatum* than either *A. granulatus* or *A. javanicus*, and greater in *A. javanicus* than *A. granulatus* (Appendix; Tables 1 and 2). Effluxes of sodium chloride were significantly greater ($P < .05$) in *A. granulatus* than *A. javanicus*, and greater in *A. javanicus* than *E. tentaculatum* (Table 2). Influx and efflux rates of sodium chloride were not significantly different ($P > .05$) in *A. javanicus*; however, efflux of sodium chloride exceeded influx in *A. granulatus* and influx exceeded efflux in *E. tentaculatum*.

Histological Examinations

The basic histology of the epidermis and dermis of *A. granulatus* and *A. javanicus* differs little from the integument of most lepidosaurs. The most notable feature is the cornified outer layer of the epidermis, and this is dealt with in detail in the microdermatoglyphics section.

The β-keratin cuticle is underlain by the tightly packed mesos-layer which in turn is underlain by α -keratin (Figures 3A and 3B). Underlying the α -keratin is an area of stratum germinativum containing proliferative keratohyaline cells which restore the original cuticle (Figure 3B).

The dermis contains varying amounts of melanin (Figure 3B), and each scale displays an associated band of muscle tissue on both the leading and trailing edges (Figures 4A and 4B). Each individual scale is associated with a blood vessel in which erythrocytes are clearly visible (Figures 6B and 7A). Striated muscle of the dermis is typical, and is associated with areas of collagen fibers (Figures 5A, 5B, and 6A). Nervous tissue with associated blood vessels is present (Figure 8), as are pressure sensitive pacinian corpuscles (Figure 7B).

The average thickness of the cornified layer was found to be 10μ for both A. granulatus and A. javanicus, and that of E. tentaculatum was 20μ .

Microdermatoglyphics

One of the most outstanding features unique to the Acrochordidae is the topography of the scalation. The term acrochordate ("warty") is applied to the general appearance of this genus.

Acrochordus granulatus differs from A. javanicus in that the scales of the former are more rounded (Figure 13A) as opposed to the trilobate form seen in those of A. javanicus (Figures 9 and 10A). Both species exhibit an echinate configuration with a prominent sharp spine (Figures 11A and 13A, B, C). Both species display smaller echinulate spines (Figures 10A and 10B) and irregular spines which anastamose into a reticulum (echinoreticulate) (Figures 12A and 14B). At the crest of each scale a sensory dome is present, and sensory bristles are evident at the apex of each dome (Figures 11A and 14A).

The cuticle of E. tentaculatum is vastly different. Each trapezodial scale exhibits a prominent keel (Figures 15A and 16A). Scales are canaliculate and channeled with longitudinal grooves (Figure 15B). There is some evidence of longitudinal parallel lines (Figure 16B), some of which anastamose into networks (Figures 16B and 16C).

The pores on the scales of acrochordids were impossible to locate because of the echinoreticulate pattern. The pores of E. tentaculatum are evident along the ridges forming the canaliculate pattern (Figures 17A and 17B). All pores located were in the size range, about 1 μ, for those given by Price (1979, 1982, 1983) for various species of snakes.

Table 1

Influx (I) and efflux (E)

of water and sodium

across shed snake skins

INFLUX (I) AND EFFLUX (E) OF WATER AND SODIUM ACROSS SHED SKINS IN μ mol/cm^{3}hr.

SPECIES, FLUX	^3H_2O $\overline{X} \pm SD$ (No.)	$^{22}NaCl$ $\overline{X} \pm SD$ (No.)
Acrochordus granulatus		
I	201.3 ± 32.8 (11)	.357 ± .36 (7)
E	175.5 ± 10.2 (10)	4.20 ± .91 (6)
Acrochordus javanicus		
I	205.4 ± 21.1 (10)	.508 ± .36 (5)
E	191.1 ± 32.8 (10)	1.04 ± .59 (6)
Erpeton tentaculatum		
I	64.2 ± 8.80 (9)	1.54 ± .22 (6)
E	54.5 ± 3.04 (9)	0.28 ± .20 (6)

Table 2

Relative Influx and Efflux

of Sodium Chloride and

water in *Acrochordus granulatus*, *Acrochordus javanicus*,

and *Erpeton tentaculatum*.

RELATIVE INFLUX AND EFFLUX OF

SODIUM CHLORIDE AND WATER

IN THE SNAKES

Acrochordus granulatus (Ag), *Acrochordus javanicus* (Aj) and

Erpeton tentaculatum (Et)

ISOTOPE	INFLUX	EFFLUX
$^{3}H_2O$	Ag > Aj > Et	Aj > Ag > Et
$^{22}NaCl$	Et > Aj > Ag	Ag > Aj > Et

Figure 1

Bar plot of water flux across shed snake skins

^3H_2O FLUX ACROSS SHED SNAKE SKINS

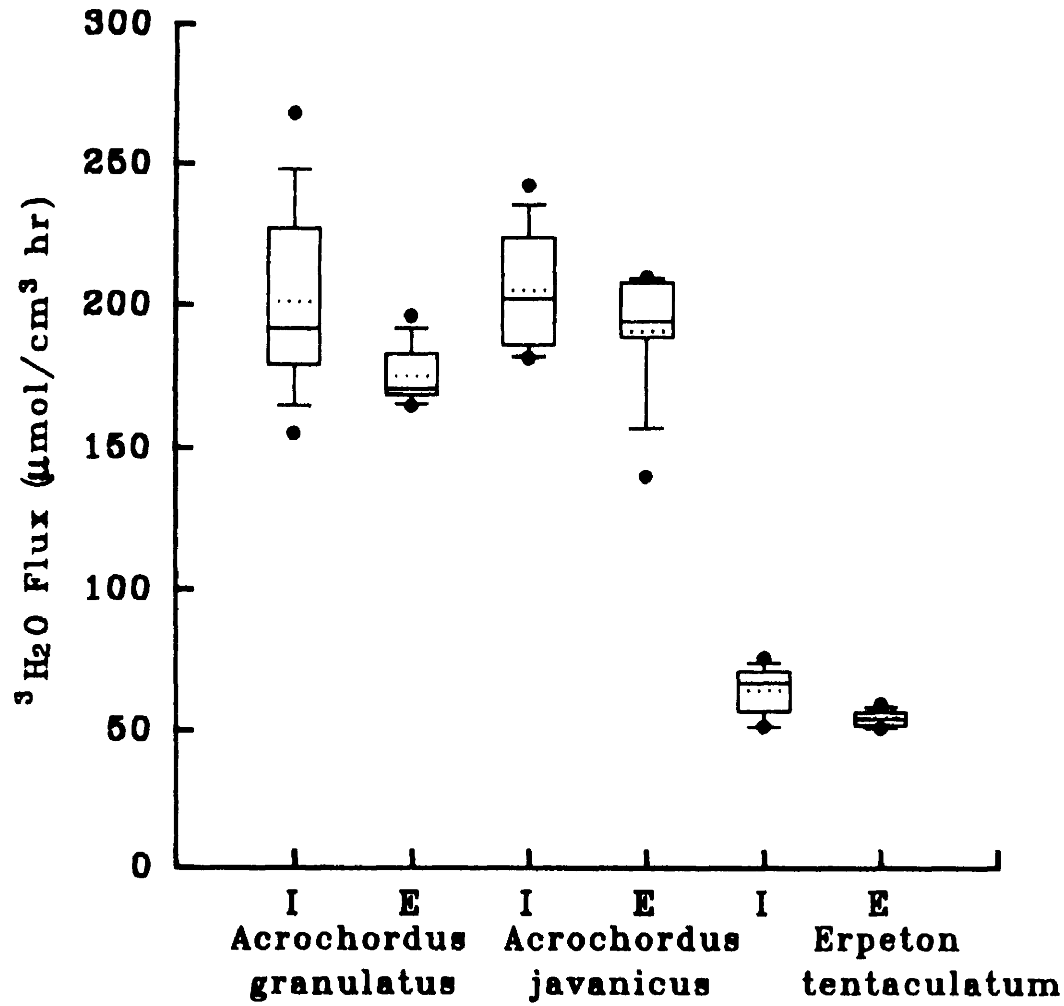


Figure 2

Bar plot of sodium flux

across shed snake skins

^{22}NaCl FLUX ACROSS SHED SNAKE SKINS

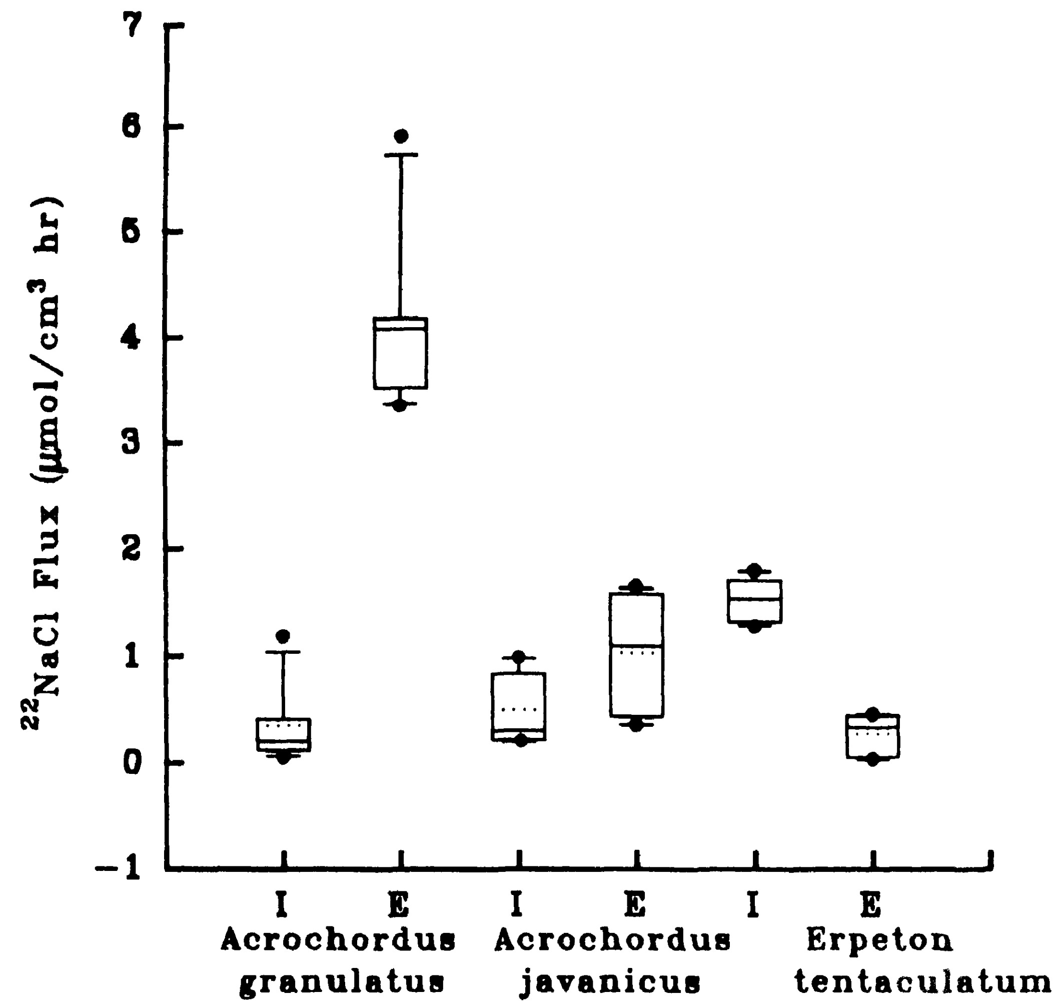


Figure 3

A. Section through _stratum corneum_ of _Acrochordus granulatus_. Spines (S) and mesos-layer (ML) clearly visible. HH&E stain.

B. Section through the skin of _Acrochordus javanicus_. _Stratum corneum_ (SC), melanin (M), and _stratum germinativum_ (SG) with keratohyaline cells (KC) in proliferative region. HH&E stain.

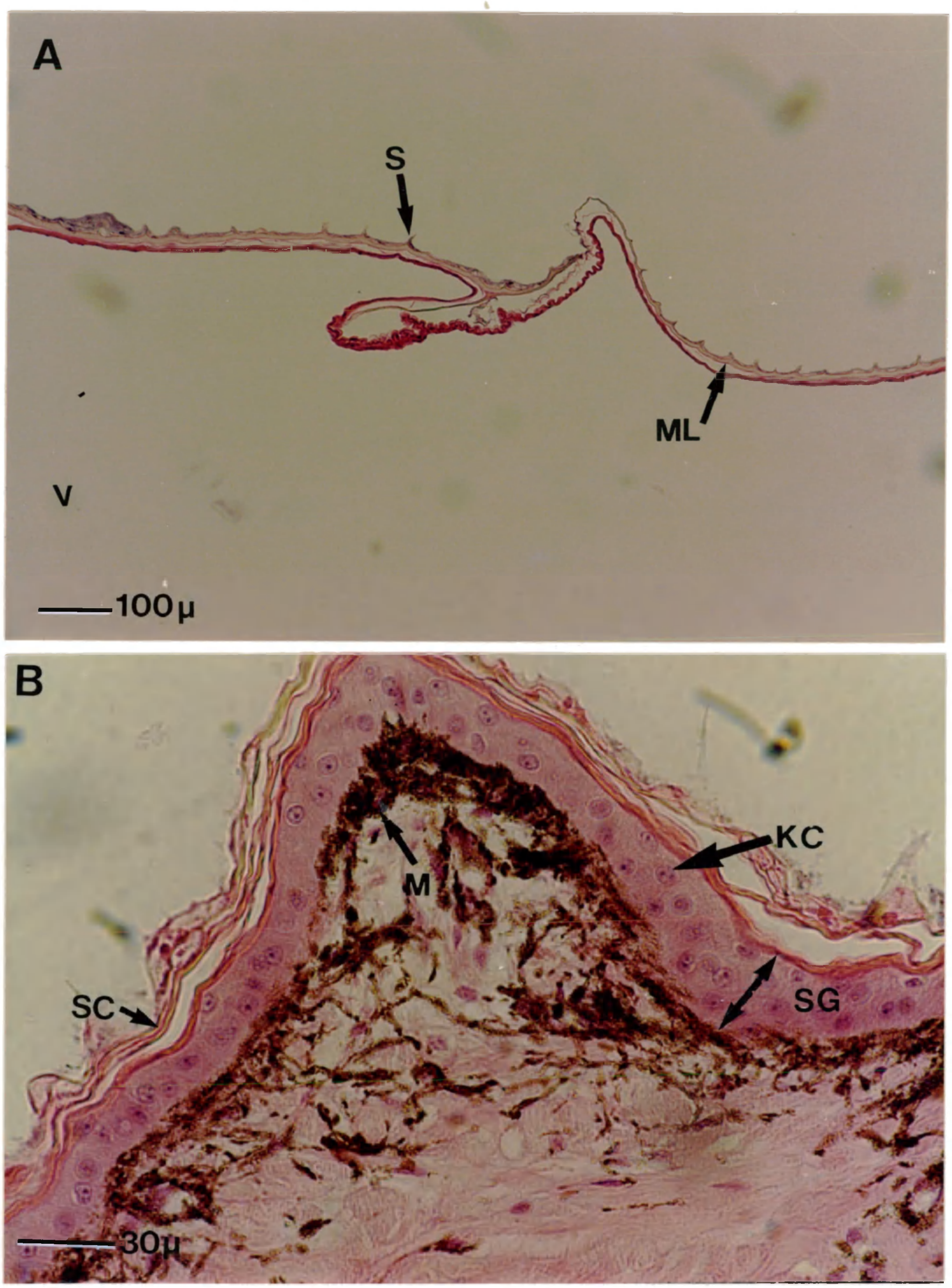

A
S
ML
V
100µ
B
KC
M
SC
SG
30µ

Figure 4

A. Section through skin of *Acrochordus granulatus*. Muscle fibers (MF) about each scale. Masson's stain.

B. Muscle fibers (MF) about each scale of *Acrochordus granulatus* in closer view. Masson's stain.

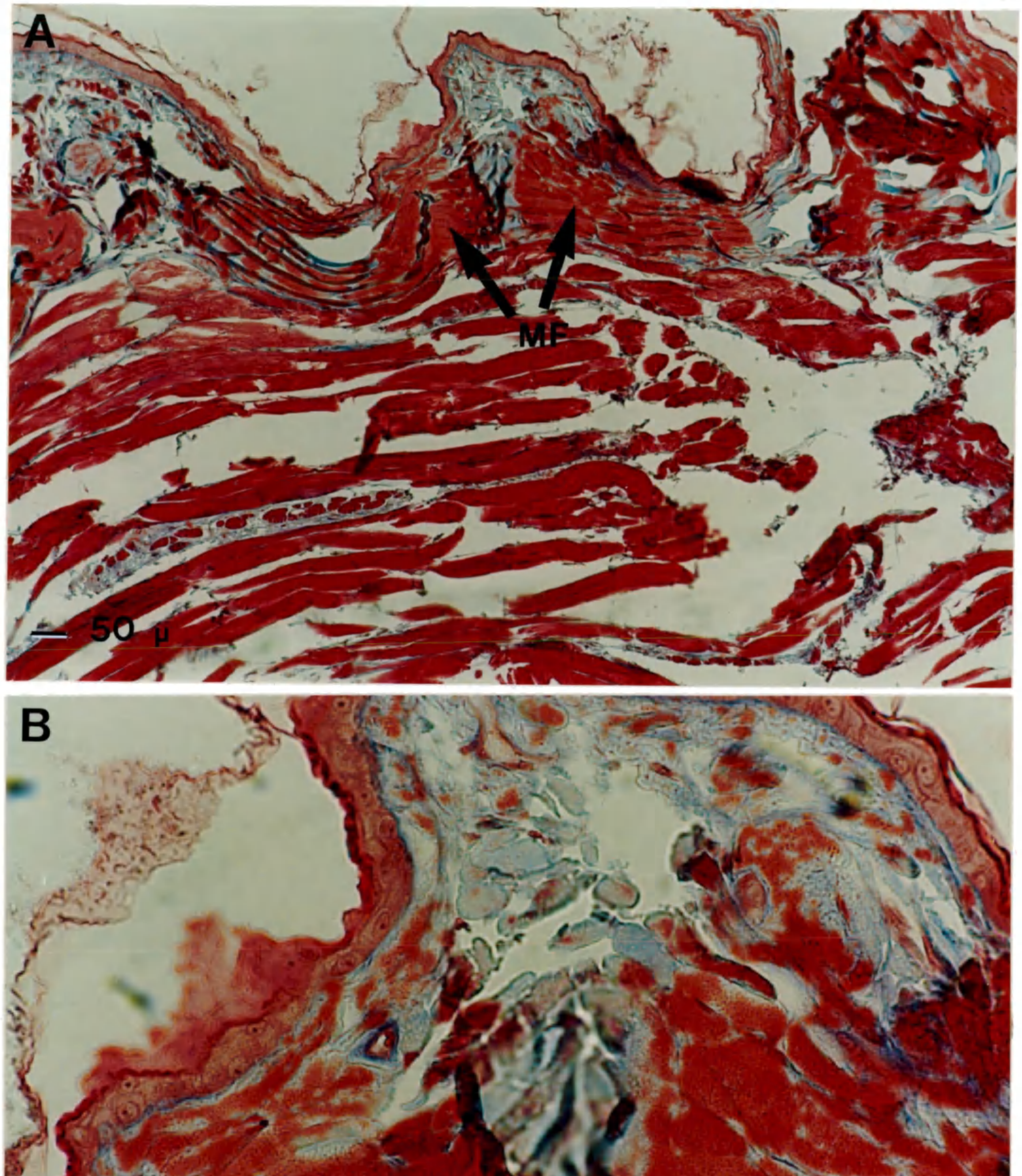

A
MF
50 μ
B
MF
30 μ

Figure 5

A. Section through the skin of Acrochordus granulatus showing connective tissue (CT) associated with striated muscle. Masson's stain.

B. Striated muscle (SM) of Acrochordus javanicus. HH&E stain.

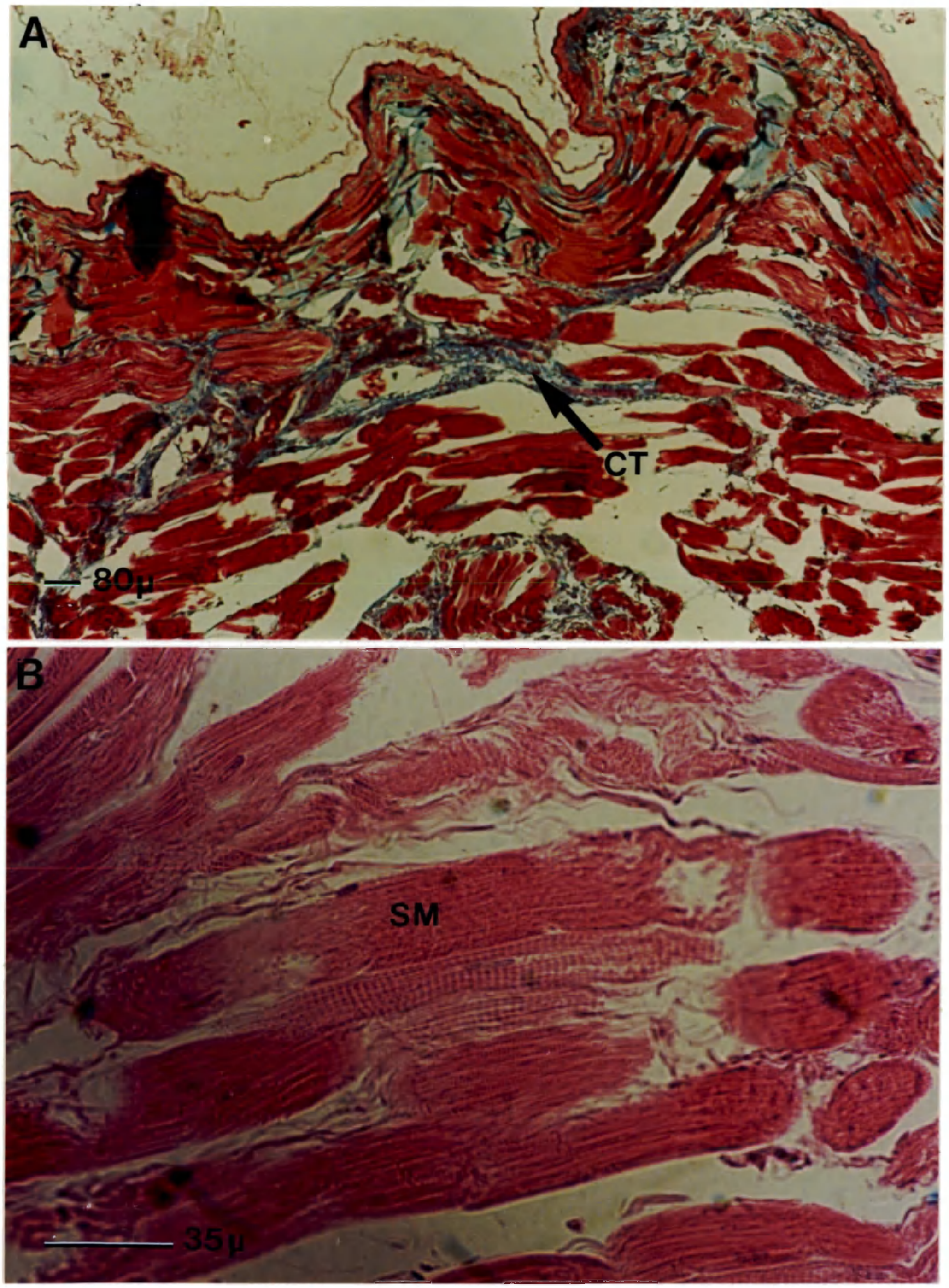

A
CT
80µ
B
SM
35µ

Figure 6

A. Striated muscle (SM) and connective tissue (CT) in *Acrochordus granulatus*. Masson's stain.

B. Scale tips of *Acrochordus granulatus*, each with associated blood vessel (BV). HH&E stain.

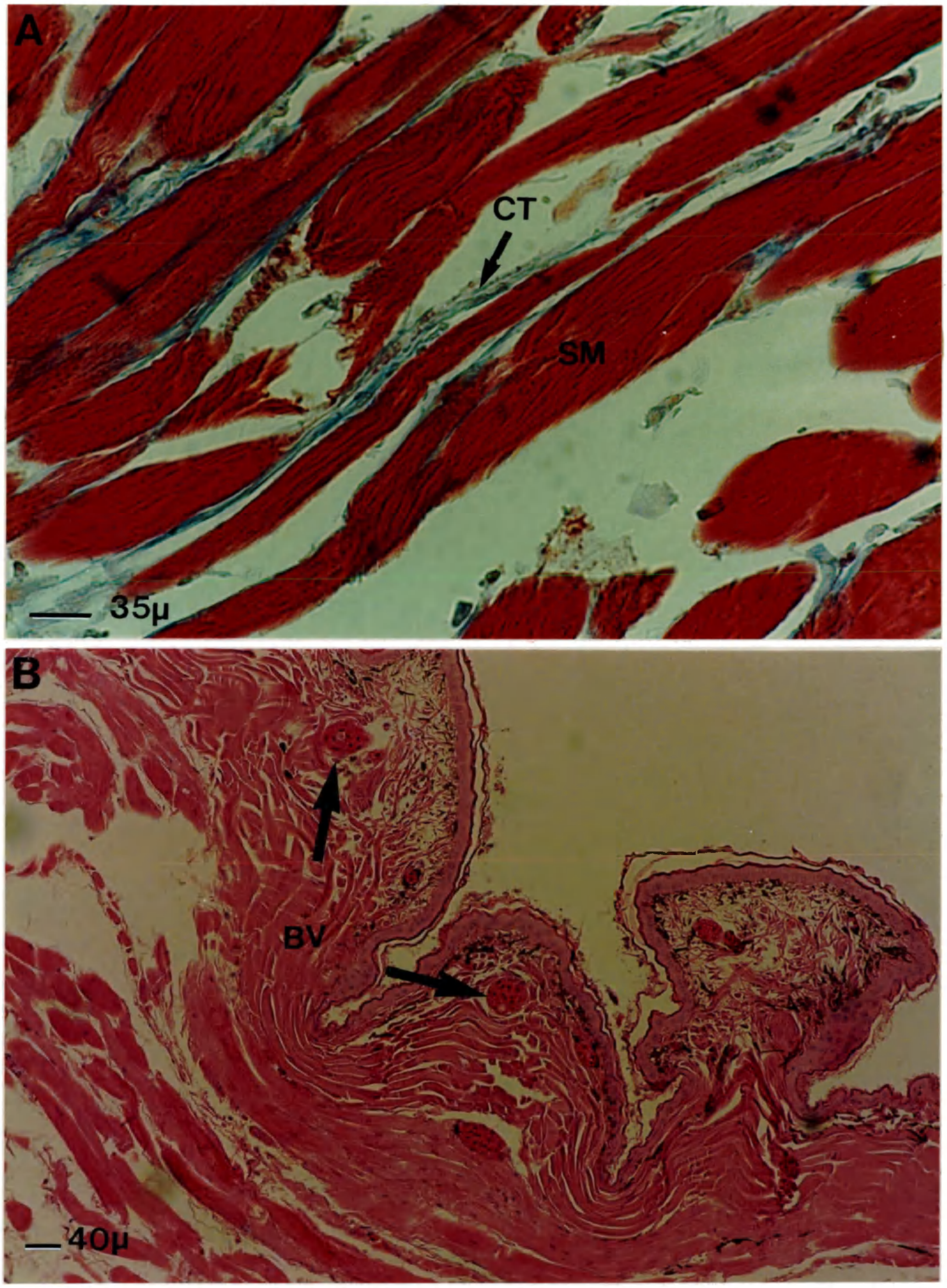

A
CT
SM
35μ
B
BV
40μ

Figure 7

A. Blood vessel (BV) with nucleate red blood cells (RBC) in the skin of Acrochordus granulatus. HH&E stain.

B. Pressure sensitive Pacinian corpuscle (PC) and red blood cells in Acrochordus javanicus. HH&E stain.

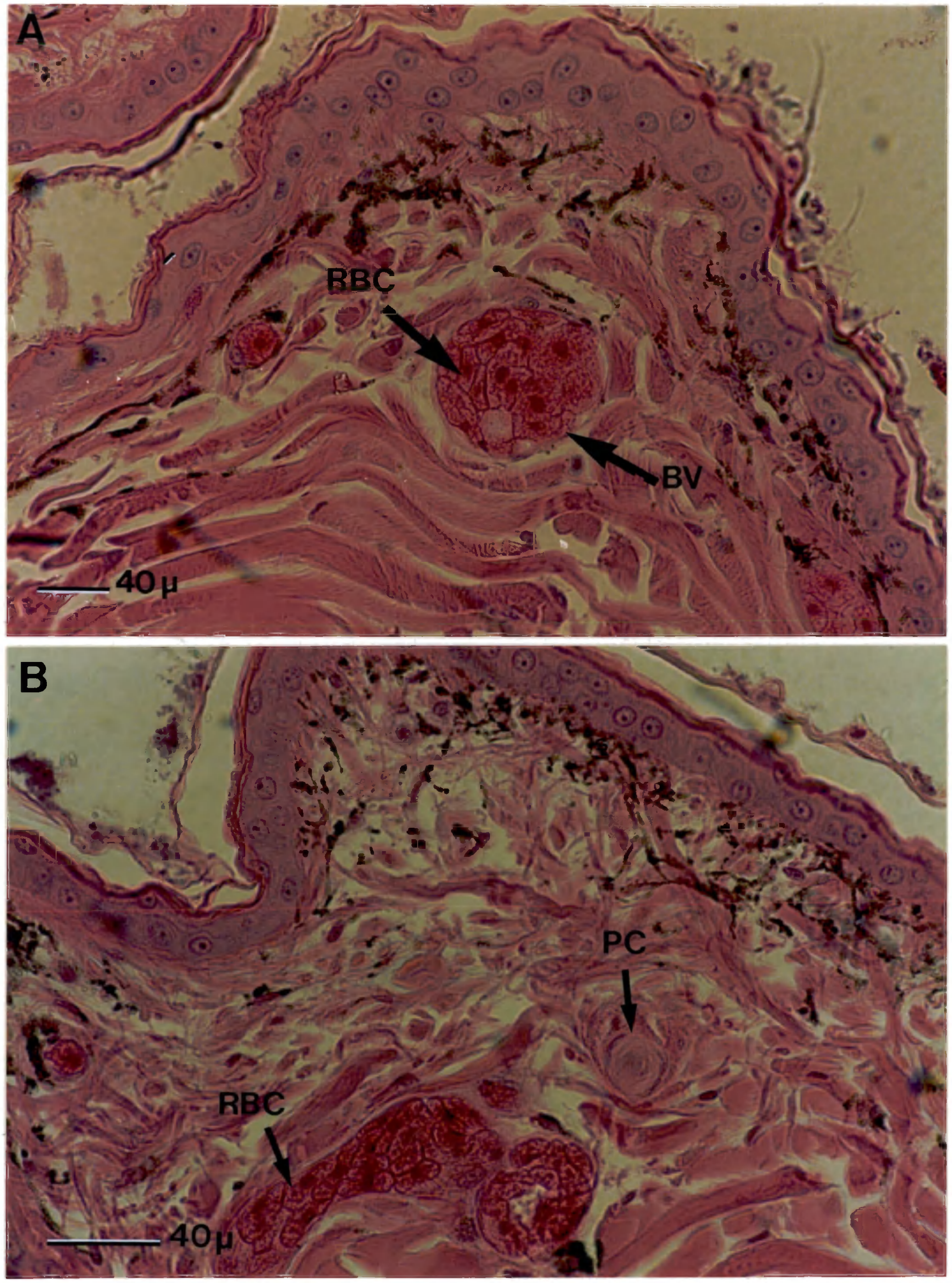

A
RBC
BV
40 µ
B
PC
RBC
40 µ

Figure 8

Red blood cells (RBC) in vessel associated with nerve tissue (NT) in *Acrochordus granulatus*. HH&E stain.

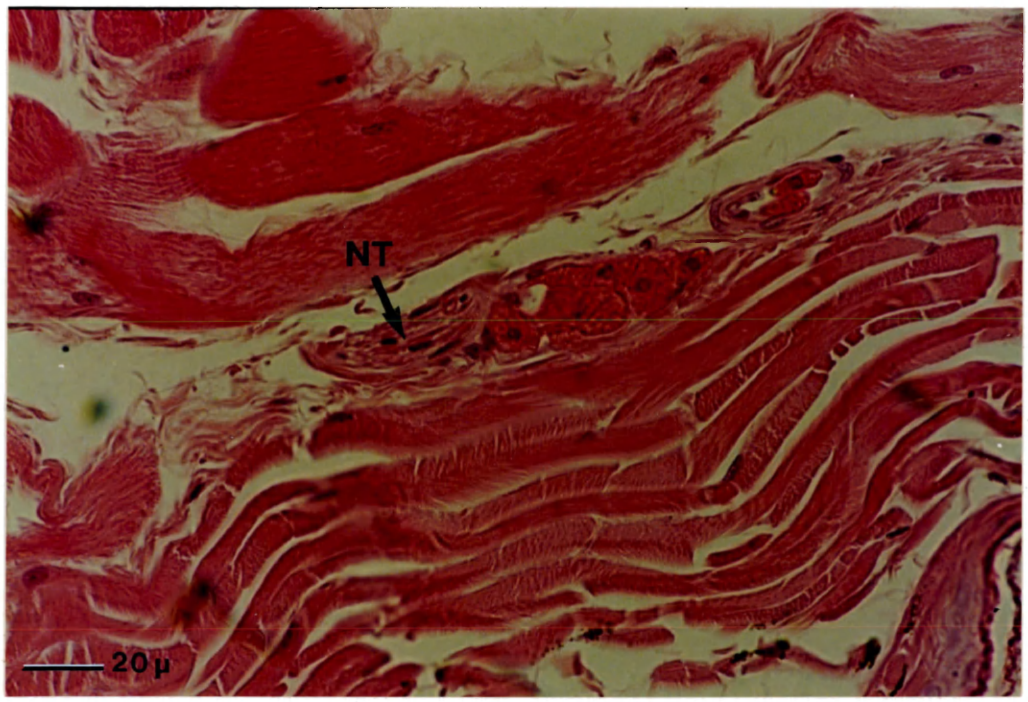

NT
20 µ

Figure 9

Dorsal scale of Acrochordus javanicus showing trilobate form. x10.

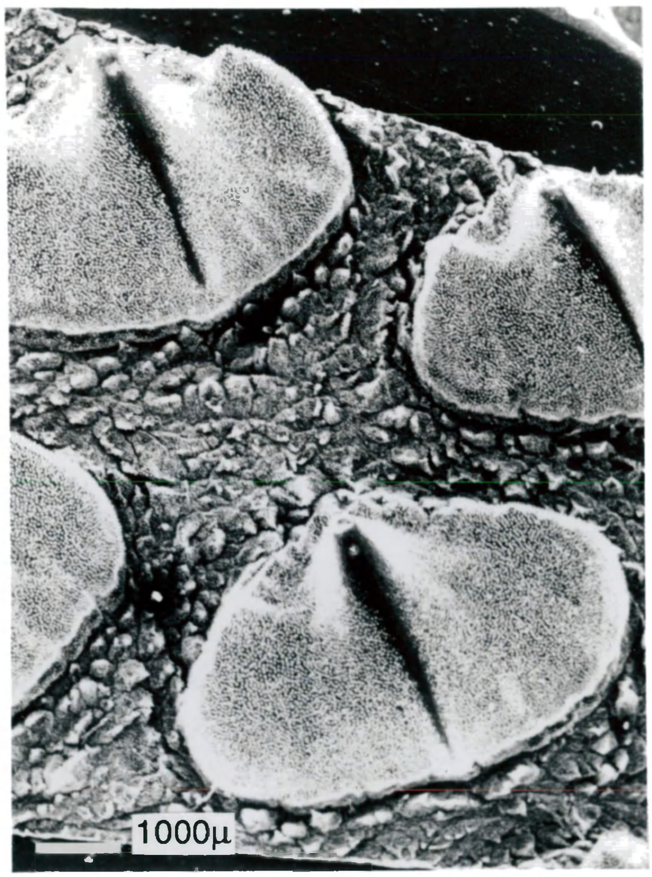

1000µ

Figure 10

A. Closeup of trilobate form with leading edge keel (K) and sensory dome (SD) in _Acrochordus javanicus_. x156.

B. Echinulate spines (ES) of _Acrochordus javanicus_. x800.

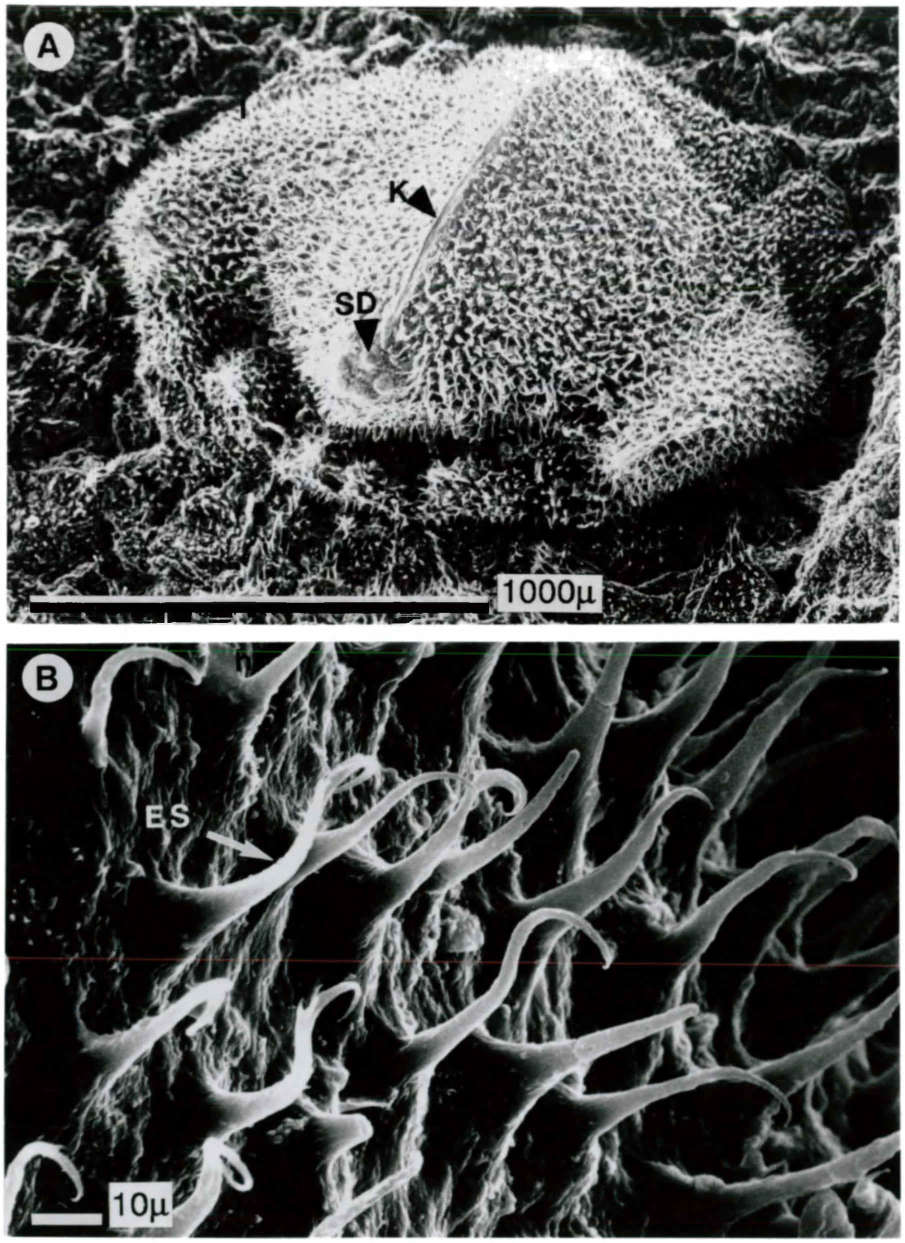

A
K
SD
1000µ
B
ES
10µ

Figure 11

A. Sensory dome (SD) with sensory spine (SS) in *Acrochordus javanicus*. x380.

B. Underside of scale of *Acrochordus javanicus* showing canal (C) leading to sensory dome. x100.

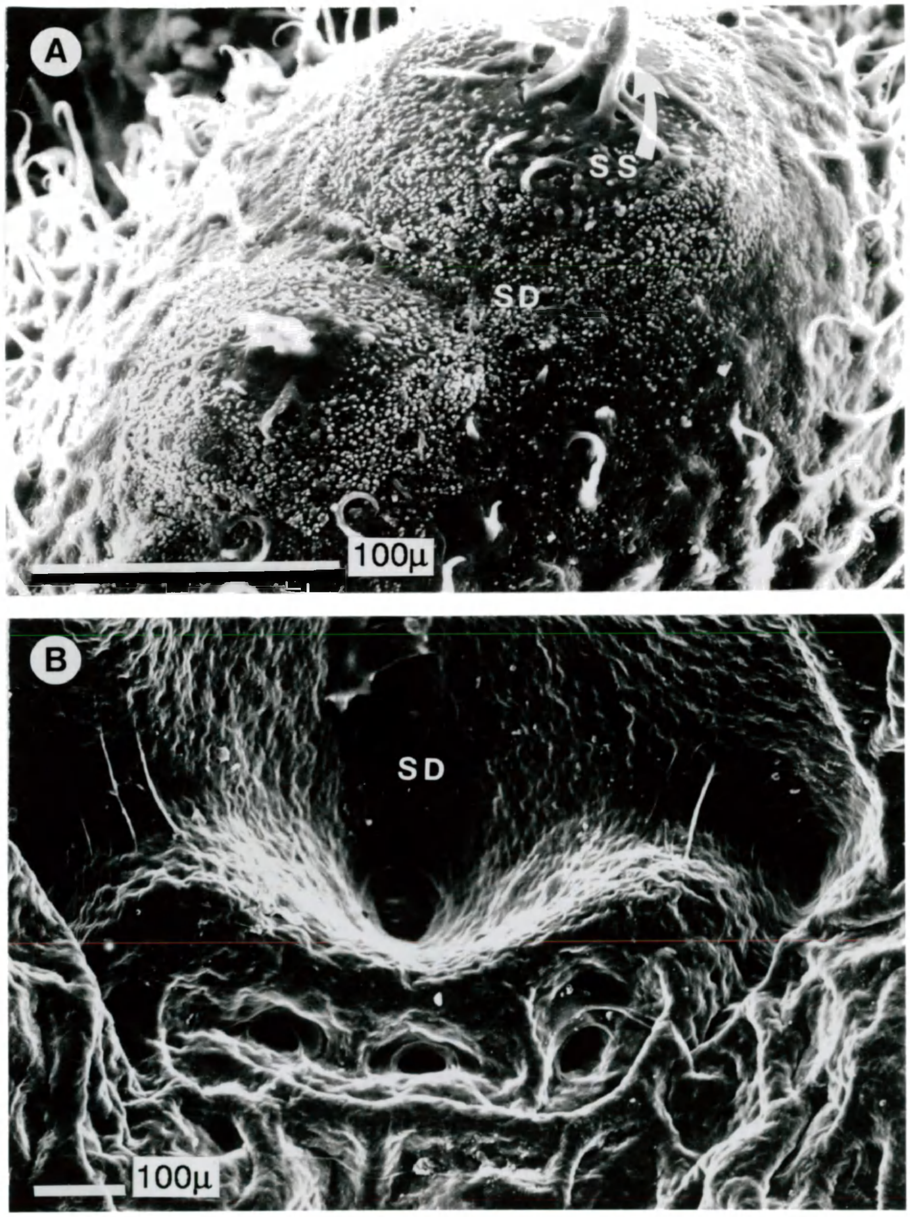

A
SS
SD
100μ
B
SD
100μ

Figure 12

A. Echinulate spines (ES) forming echinoreticulate pattern in *Acrochordus javanicus*. x8000.

B. Skin between scales of *Acrochordus javanicus* viewed from below. x900.

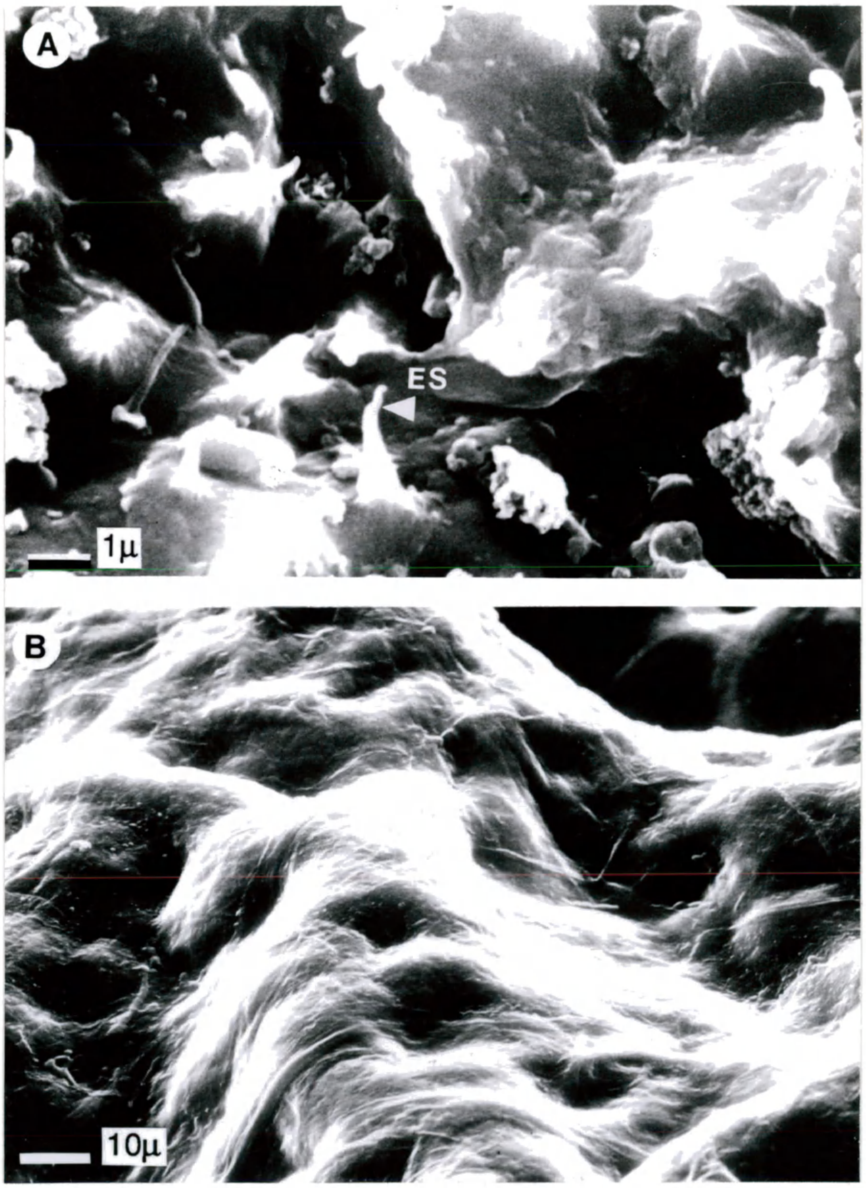

A
ES
1μ
B
10μ

Figure 13

A. Basic topography of the skin of _Acrochordus granulatus_ showing rounded form of scales, each with a prominent spine. x59.

B. Single scale of _Acrochordus granulatus_ with sensory dome (SD). View from trailing edge. x110.

C. Sensory dome (SD) and sensory spine (SS) of _Acrochordus granulatus_ viewed from above. x100.

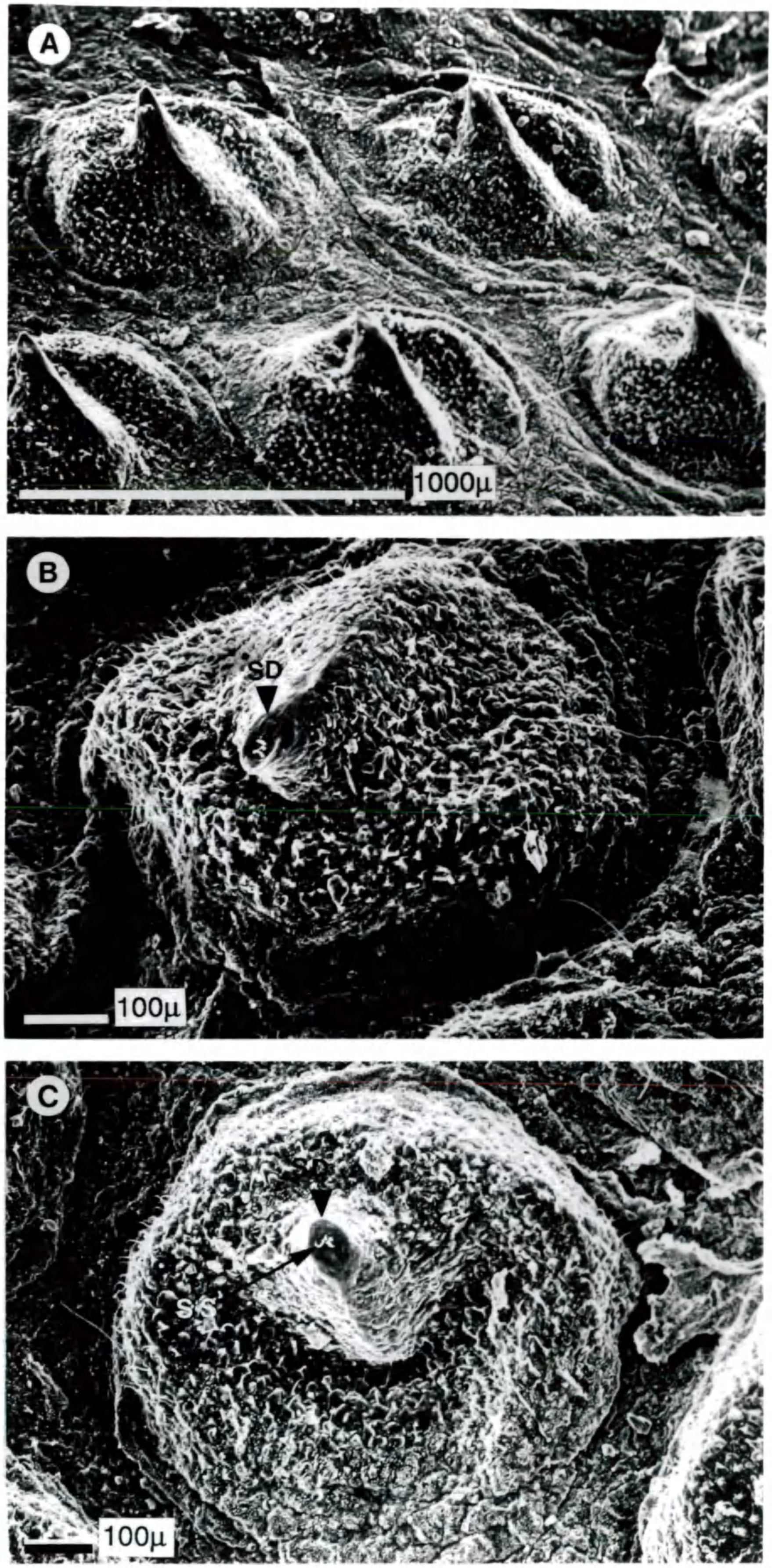

A
1000μ
B
SD
100μ
C
SS
100μ

Figure 14

A. Closeup of sensory dome (SD) and sensory spine (SS) of *Acrochordus granulatus*. x800.

B. Echinulate spines (ES) in *Acrochordus granulatus* forming echinoreticulate pattern. x800.

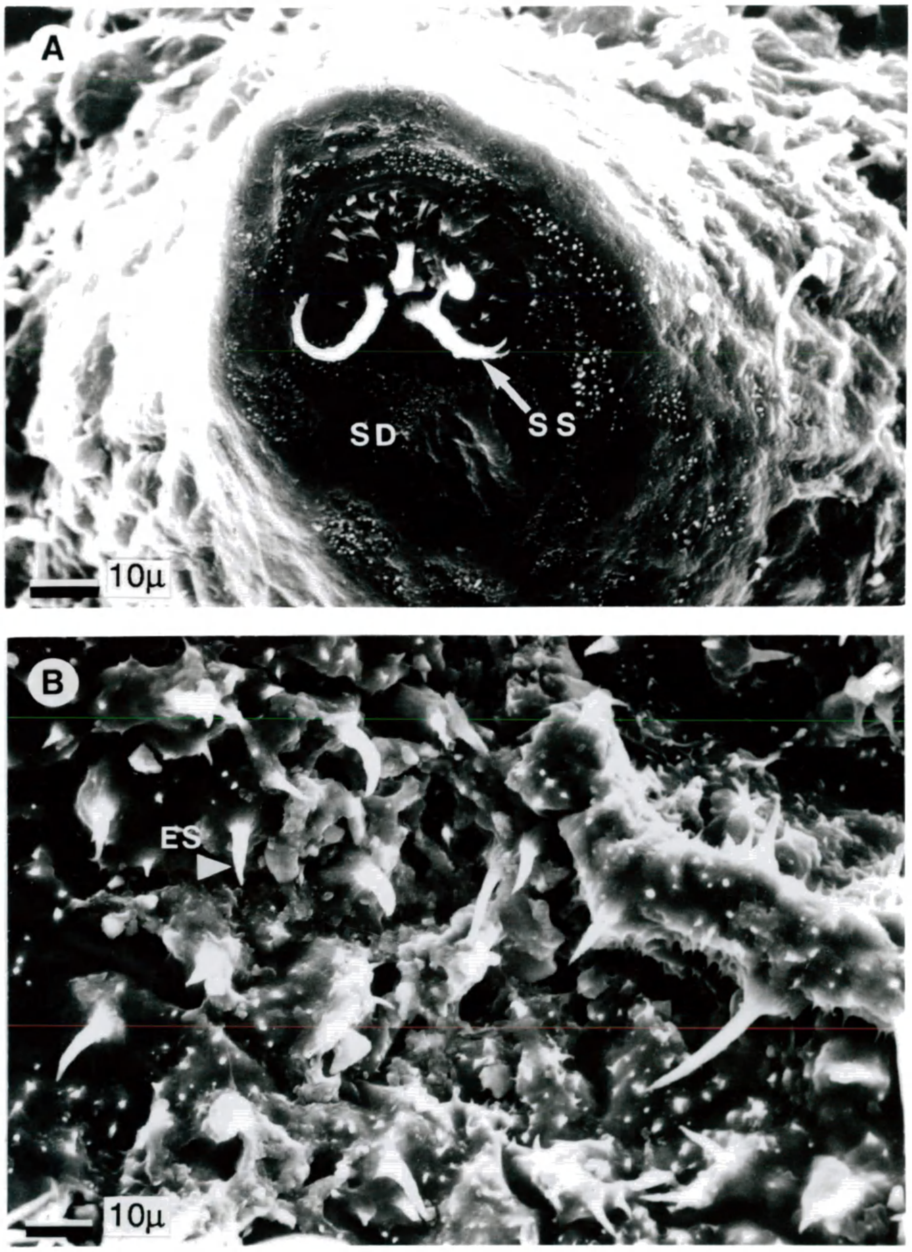

A
SS
SD
10μ
B
ES
10μ

Figure 15

A. Scales of _Erpeton_ _tentaculatum_ with prominent keel (K). x21.

B. Canaliculate ridge (CR) in _Erpeton_ _tentaculatum_. x40.

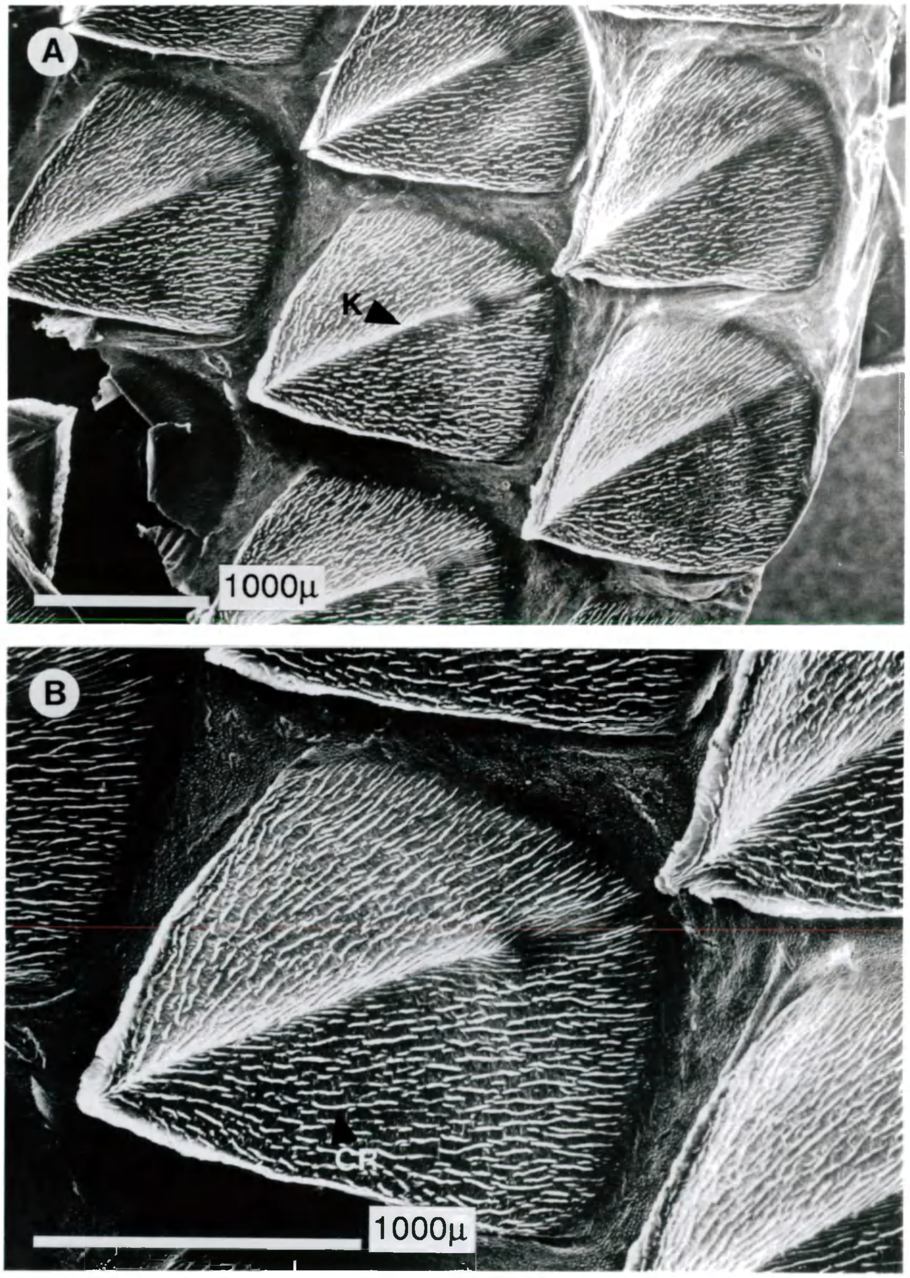

A
K
1000μ
B
CR
1000μ

Figure 16

A. Canaliculate pattern along keel (K) in _Erpeton tentaculatum_. x190.

B. Canaliculate pattern with anastamosing ridges (AR) in _Erpeton tentaculatum_. x330.

C. Anastamosing ridges (AR) with canals (C) and associated pits (PT) in _Erpeton tentaculatum_. x800.

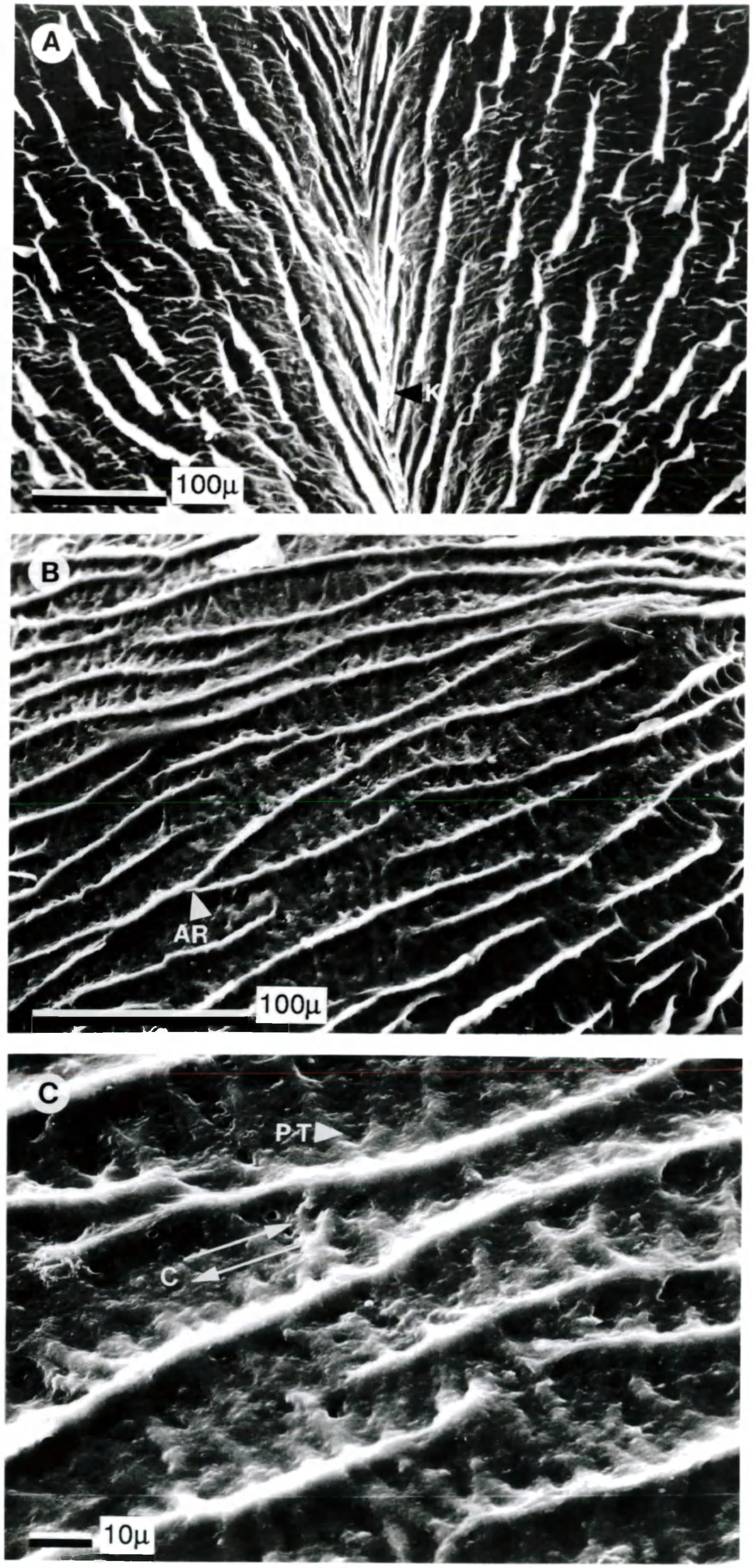
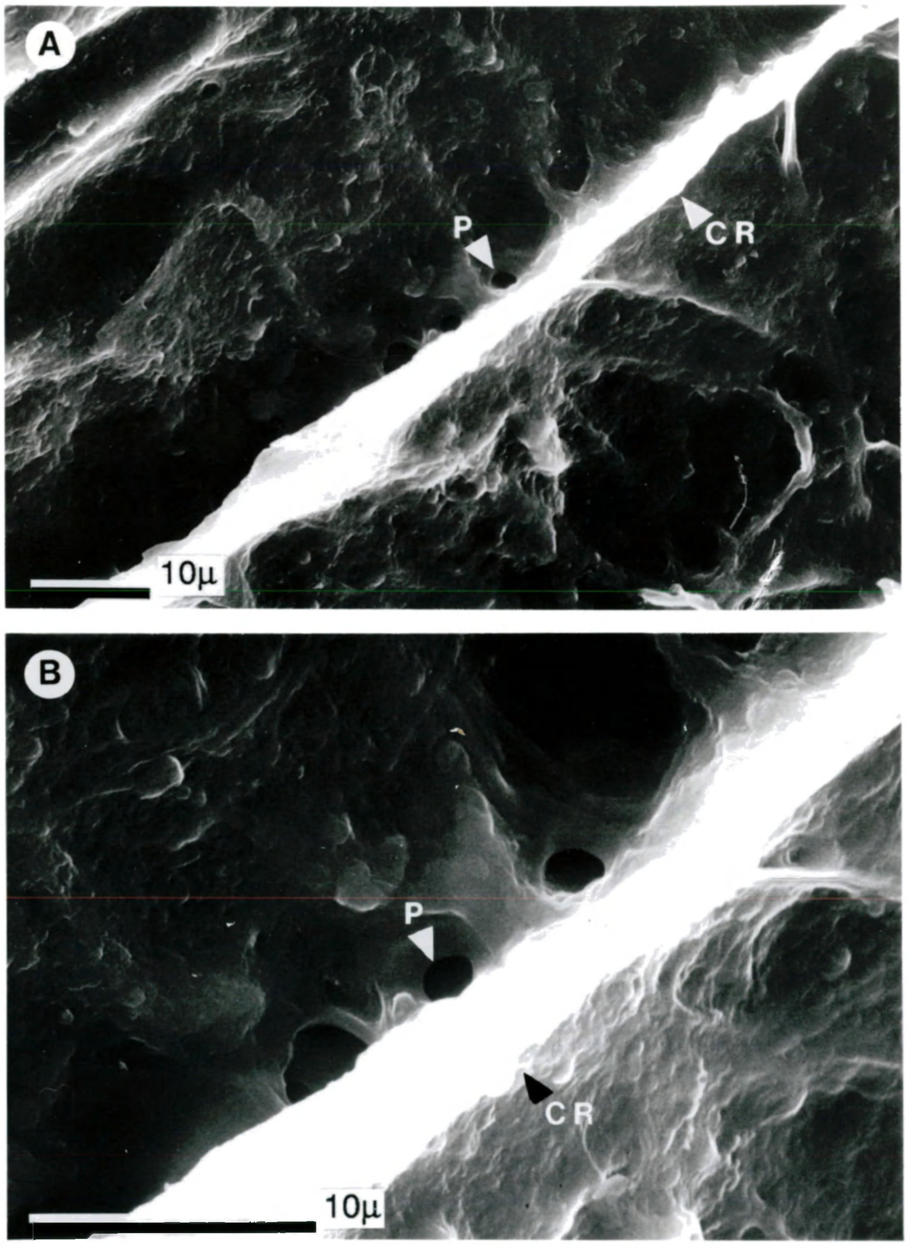

A
K
100μ
B
AR
100μ
C
P-T
C
10μ

Figure 17

A. Canaliculate ridge (CR) with pores (P) in *Erpeton tentaculatum*. x1400.

B. Closeup of canaliculate ridge (CR) with pores (P) in *Erpeton tentaculatum*. x3500.


A
P
CR
10μ
B
P
CR
10μ

DISCUSSION

Skin Morphology

The skin of all vertebrates is a heterogeneous multimembrane system with diffusion pathways for specific molecules (Hahn-Bereiter et al., 1986). The skin of lepidosaurs is unique in that it contains a complex stratified epidermis that is periodically regenerated and, in the case Serpentes, shed in its entirety (Landmann, 1979). The acrochordids examined revealed an epidermis and dermis histologically typical of snakes (Lillywhite and Maderson, 1982), but differing in the juxtaposed scalation.

Microdermatoglyphics

The conical, juxtaposed scales of acrochordid snakes is a condition more typical of early tetrapods (McDowell, 1979) and has been lost in all lepidosaurs with the exception of the Acrochordidae and the Bornean Lanthanotidae (Sauria). the importance of scale morphology of *Acrochordus* in tactile stimulation has recently been suggested by Banks (1989) and much earlier by Schmidt (1918). In view of the prey capture methods of the Acrochordidae (grasp and constrict) (Dowling, 1960), I suggest that the scales play an integral role that is two-fold. Having conical scales with sensory bristles at the tips is an aid in the detection of fishes in close surroundings where the acrochordids act as "sit-and-wait" predators. Secondly, once the prey has been detected and grasped, the spiny morphology of the scale acts to hold slippery prey during the constricting process. The present findings strongly support the earlier inference of Gans and Baic (1977) that scale morphology is an adaptation to the environment for Acrochordidae. Microdermatoglyphics examination of _E. tentaculatum_ revealed scale topographies characteristic of many snakes.

Of all serpents, only two primitive snake families, the Acrochordidae and the Uropeltidae, are known to have scale topographies indicative of adaptations to specific habitats or behaviors. More recently evolved Serpentes display various arrays of topographies, none of which have yet been correlated with adaptations to habitat (Price, 1979, 1982, 1983).

Salt and Water Balance

Osmoregulation in reptiles is of particular interest because of the wide range of ecological conditions tolerated by this class of animals. Gans _et al_ (1968) noted different interspecific integumental permeabilities to water and sodium in snakes, and the species examined in this study exhibit water and sodium permeabilities generally correlated with marine, estuarine, and freshwater environments (Dunson and Robinson, 1976). Water transport across the _stratum corneum_ in the genus _Acrochordus_ reflects different habitat utilization even in closely related species. Influx of water is higher in the marine _A. granulatus_ than in _A. javanicus_, an estuarine species. Thus _A. granulatus_ maintains electrolyte concentrations hypo-osmotic to its medium whereas _A. javanicus_ maintains electrolyte concentrations which are iso-osmotic. _Erpeton tentaculatum_, a species inhabiting slow-moving freshwater streams and ponds, exhibits low water permeability, and so remains hyper-osmotic to its medium.

Just as the _stratum corneum_ is a major avenue of water flux in snakes, so the outer integument is the primary barrier to sodium movement. Influx of sodium in _A. granulatus_ is comparatively very low in proportion to efflux, a situation typical of snakes living in fully marine environments (Dunson and Dunson, 1973). Since _A. javanicus_ does not possess a salt gland (Dunson, 1984), some efflux of sodium must be dealt with by the skin, as indicated by its relatively high efflux rates. _Erpeton tentaculatum_ has the highest sodium uptake of all species used in these experiments, and its efflux rates of sodium are much lower. Movement of sodium across the skin of this species is adaptive in snakes inhabiting fresh water, as most homolopsines do (Banks, 1989). In its freshwater habitat, sodium is essentially a precious commodity, the main source being teleost food-fishes which are notably low in their concentrations of sodium chloride (Cohen, 1975). Thus the ability of _Erpeton_ to retain sodium through integumentary mechanisms has selective advantages in its freshwater habitat.

For the estuarine and marine species used in this study, water loss to the environment is a greater problem than sodium gain from it, and in both freshwater and saltwater species, the exchange of molecules and ions across the _stratum corneum_ reflects osmotic balance and habitat utilization.

Since the early studies of Pettus (1963), the _stratum corneum_ has provided a tool for the studies of passive transport of molecules across the skin, which reflects physiological adaptation to specific ecological conditions. The importance of the underlying tissue in water and ion transport should not be disregarded (Schafer and Andreoli, 1972). Cohen (1975) has suggested that the permeability of the _stratum corneum_ changes as a function of progress into the shedding cycle. Dunson and Freda (1985) agree that it is incorrect to assume that a given species has constant values of permeability through the skin. Future studies will require comparative investigations of specific transport pathways, their rate limiting resistances, and the interactions as well as individual regulation of transported molecules across whole intact skins.

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APPENDIX

Flux values of sodium

and water for _Acrochordus_

granulatus

INFLUX (I) AND EFFLUX (E) OF WATER AND SODIUM

ACROSS THE SHED SKIN OF *Acrochordus granulatus*

IN μ mol/cm^{3}hr

WATER (^3H_2O)		SODIUM ($^{22}NaCl$)	
I	E	I	E
155.1	165.0	.060	3.37
171.9	166.5	.110	3.54
177.8	169.0	.188	4.08
184.5	169.1	.210	4.10
190.0	171.3	.287	4.19
191.9	171.3	.458	5.91
199.3	176.1	1.19	
205.3	183.0		
234.5	187.7		
235.2	196.2		
268.0			

Flux values of sodium

and water for *Acrochordus*

javanicus

INFLUX (I) AND EFFLUX (E) OF WATER AND SODIUM

ACROSS THE SHED SKIN OF *Acrochordus javanicus*

IN μ mol/cm^3hr

WATER (^3H_2O)		SODIUM ($^{22}NaCl$)	
I	E	I	E
181.7	140.0	.210	.359
182.5	174.4	.232	.442
186.1	188.7	.315	.707
191.2	189.2	.790	1.49
200.3	194.1	.988	1.59
204.2	194.4		1.66
212.2	201.9		
224.0	208.0		
229.1	209.6		
242.2	209.6		

Flux values of sodium

and water for *Erpeton tentaculatum*

INFLUX (I) AND EFFLUX (E) OF WATER AND SODIUM

ACROSS THE SHED SKIN OF *Erpeton tentaculatum*

IN μ mol/cm^{3}hr

WATER (^3H_2O)		SODIUM ($^{22}NaCl$)	
I	E	I	E
51.3	50.6	1.29	0.04
51.7	51.5	1.33	0.06
58.5	51.9	1.40	0.25
62.8	52.5	1.70	0.43
66.8	54.3	1.72	0.45
69.0	56.4	1.81	0.46
70.8	56.6		
71.6	56.9		
75.7	59.5		

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