

## Morphology and Ultrastructure of Possible Integumentary Sense Organs in the Estuarine Crocodile (*Crocodylus porosus*)

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**ABSTRACT** The skins of crocodylids and gavialids can be distinguished from those of alligatorids by the presence of darkly pigmented pits, known as integumentary sense organs (ISOs), on the postcranial scales. The structure of ISOs, in *Crocodylus porosus*, was studied using light microscopy and scanning and transmission electron microscopy. The stratum corneum of the epidermis in the area of the ISO is thinner, while the stratum germinativum is thicker, relative to other regions of the integument. Beneath the epidermal layer the ISO region has a paucity of collagen fibers relative to the rest of the dermis. Widely dispersed fibrocytes, nerve terminals, and chromatophores occur throughout the ISO region of the dermis, but these elements are concentrated in the area immediately beneath the stratum germinativum in the ISO region. The morphology of the ISOs suggests that they are sensory organs. It has traditionally been assumed that sensory organs on the amniote integument have a mechanosensory function. However, alternate functional interpretations of this structure are possible, and a resolution awaits further work.

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Integumentary pits are present, one per scale, on the postcranial scales of some crocodylians, and these have been used extensively as taxonomic characters in the identification of crocodylian skins (King and Brazaitis, '71; Wermuth and Fuchs, '78; Brazaitis, '87). The pits are present on the postcranial scales of crocodylids (Fig. 1a) (including *Tomistoma*) and gavialids, but are absent from the postcranial scales of alligatorids (Fig. 1b). The presence or absence of pits can therefore be used to distinguish alligatorids from the other two crocodylian families. The pits have been referred to in the taxonomic literature as "follicle pores" (King and Brazaitis, '71), "Poren" (Wermuth and Fuchs, '78), "follicle glands" and "follicle pits" (Brazaitis, '87), and "integumentary sense organs" or "ISOs" (Brazaitis, '87). In this study, the term *ISO* will be used throughout, for reasons to be explained below.

Although the ISOs have been well studied as taxonomic characters, their structure and function are not known. Brazaitis ('87) says that they are thought to be mechanosensory, while Grigg and Gans ('93) speculate that they may be either sensory structures or secretory pores. A search of the literature reveals no detailed study of their structure or

function. In contrast, detailed morphological and ultrastructural studies have been performed of mechanoreceptors or "touch papillae" on the cranial scales of the alligatorid, *Caiman crocodilus* (von During, '73, '74; von During and Miller, '79). These touch papillae are confined to the cranial scales and are found in all crocodylians (Fig. 2). The touch papillae on the cranial scales of *Caiman crocodilus* are not the postcranial ISOs referred to in the taxonomic literature, as alligatorids lack ISOs on the postcranial scales. However, von During's work may nonetheless be the source of the idea that the ISOs have a mechanosensory function. Guibé ('70) reported that the abundance of ISOs decreases as the animal ages. The source of this information, however, is a study which compared juvenile *Crocodylus* with adult *Alligator* (Hulanicka, '13), and it seems likely that this difference may have more to do with phylogeny than with ontogeny. The objective of the present study was to investigate the general morphology and the ultrastructure of the ISOs of

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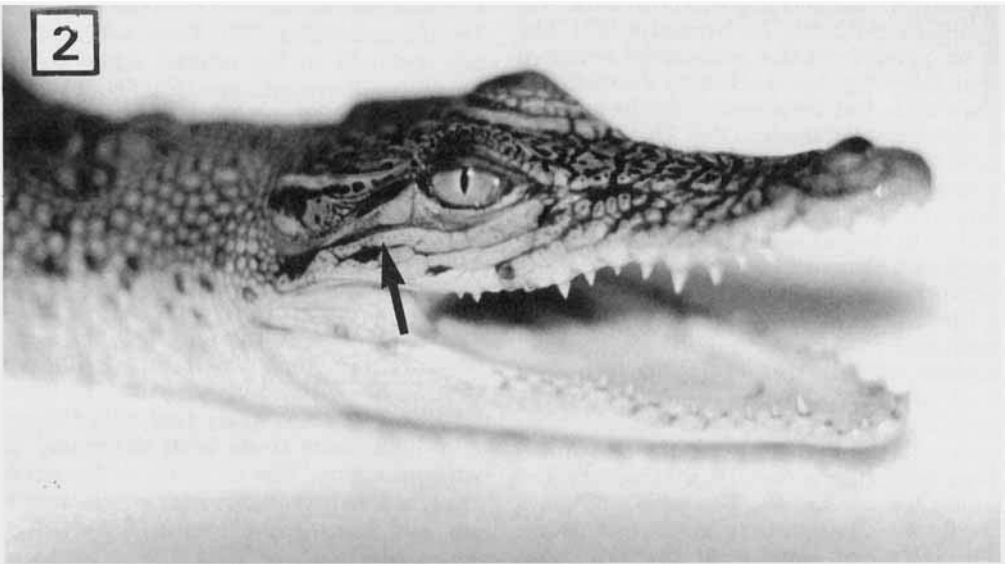
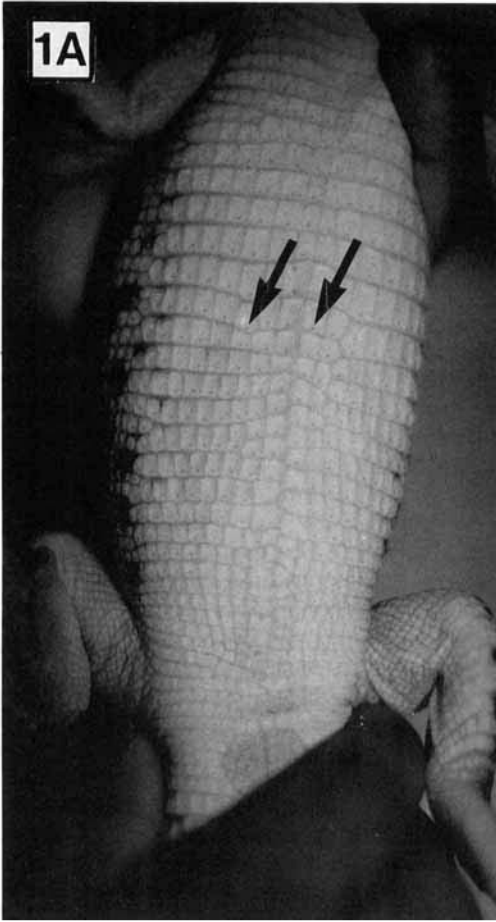


Fig. 1. **A:** Ventral scales of a crocodylid, *Crocodylus porosus*, showing the ISOs (arrows). **B:** Ventral scales of an alligatorid, *Caiman crocodilus*, which lacks ISOs.

Fig. 2. Touch papillae (sensu von During, '73) from the cranial scales of *Crocodylus porosus*. Head of a crocodile with touch papillae indicated (arrow).

*Crocodylus porosus* as one of the means we will use to determine their function.

#### MATERIALS AND METHODS

##### *Animals*

Captive-bred juvenile (400–700 g) crocodiles (*Crocodylus porosus*) were obtained from the Long Kuan Hung Crocodile Farm in Singapore, where they had been housed in fresh water in an outdoor enclosure. A total of five specimens was obtained, of which four were frozen specimens used for gross morphological studies. The fifth, a live specimen, was euthanized by cervical dislocation in order to fix tissues for electron microscopic examination.

##### *Scanning electron microscopy (SEM)*

Integument was dissected from the ventral surface of the freshly killed crocodile and transferred immediately to Bouin's solution. Following 24 h fixation, tissue was transferred to 70% ethanol. Cubes of tissue (1 mm<sup>3</sup>), each with an ISO at its center, were cut out of the ventral integument. Specimens were dehydrated in a graded series of ethanol, dried in a Sorvall 49300 critical point drying system, mounted on metal stubs, sputter-coated with gold, and observed in a Hitachi H-2500 scanning electron microscope at an acceleration voltage of 15 kV.

##### *Light microscopy and transmission electron microscopy (TEM)*

Integument was dissected from the ventral surface of the freshly killed crocodile and transferred immediately to a solution of ice-cold 2.5% glutaraldehyde in 0.1 M Millonig's phosphate buffer at pH 7.3. The tissue was cut into 1 mm cubes, each with an ISO at its center, and fixed in the above fixative for 3 h. The tissue cubes were then rinsed in the buffer, stored in the buffer for 3 days, and postfixed for 2 h in 1% OsO<sub>4</sub> in the same buffer. Tissues were dehydrated in ethanol and propylene oxide and embedded in Spurr's resin. Tissue blocks were sectioned with glass knives, using a Sorvall MT2 ultramicrotome. Semithin (0.5 μm thickness) sections were placed on glass slides and stained with 1% toluidine blue in saturated sodium tetraborate. Thin (silver) sections were cut using a diamond knife and mounted on copper grids. In some cases formvar-coated single-slot grids were used. The specimens were stained with saturated uranyl acetate and lead citrate and examined using a Hitachi H-7000 transmission electron microscope.

#### RESULTS

##### *Gross morphology*

ISOs are present on almost all the postcranial scales. The pits are darkly pigmented and are therefore most clearly visible on the large and relatively unpigmented ventral scales (Fig. 1A). However, they are also present on the darkly pigmented dorsal scales and on the very small scales surrounding the proximal ends of the limbs. There is usually one ISO on each scale, although the number occasionally varies from zero to three. The ISO is usually centered (sagittally) in the caudal third of the scale. When more than one ISO is present on a single scale, the two (or three) ISOs are positioned in line along the same transverse plane.

##### *Scanning electron microscopy*

In SEM, the outer surface of the ISO is revealed as a roughly circular opening, approximately 300 μm in diameter, in the stiff outer layer of the stratum corneum which forms a protective coating over the scales. The slightly convex surface of the epidermis of the ISO is revealed through the opening (Fig. 3A,B). At the edges of the circular opening, the outer layer of keratin flakes off in stiff sheets. When the convex surface of the ISO is viewed at high magnification (Fig. 3C), the margins between adjacent epidermal cells can be seen. The surfaces of these cells are pitted and possibly porous (Fig. 3D).

##### *Light microscopy and transmission electron microscopy*

In cross-section and at low magnification (Fig. 4A,B) the ISO is revealed as a diffuse, lightly stained pocket in the darkly stained, collagen-rich surrounding dermal tissue. The ISO occupies an ellipsoidal space in the dermis immediately underlying the circular opening in the stratum corneum proper of the epidermis. Cells in the dermal portion of the ISO are widely separated, and there are very few collagen fibers in comparison with the surrounding dermis. Those cells that are present are usually densely concentrated at the apex of the dermal sphere, in the area immediately underlying the stratum germinativum. The stratum corneum appears to have two distinct layers: an outer layer through which the underlying second layer protrudes through a circular opening. During sectioning, the outer layer tended to separate from the underlying layer and to break off the tissue block.

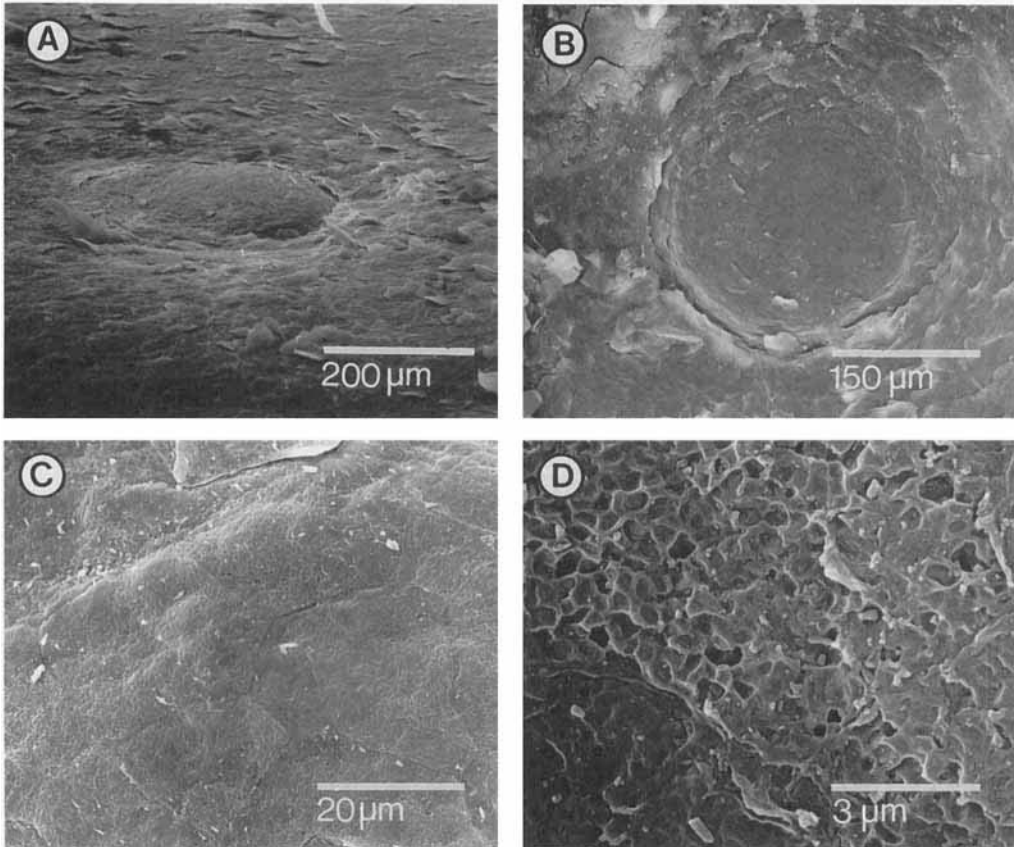


Fig. 3. SEMs of the ISOs from the ventral scales of *Crocodylus porosus*. A: Ventro-lateral view. B–D: Ventral view.

The cells of the ISO beneath the epidermal layer are widely dispersed among a few collagen fibers and extensive ground substance of extracellular matrix. Three cell types were observed in the dermal region of the ISO, and all of these are most numerous at the apex of the dermal region of the ISO, in the area immediately underlying the stratum germinativum of the epidermis (Figs. 4A, 5). Fibroblasts (Fig. 6) are the most abundant cell type, but melanocytes are also common. All cells are often found close to nerve terminals (Fig. 7), but structural support for the nerve terminals may be provided by the attenuated processes of fibroblasts (Fig. 7). There are two types of chromatophores: iridocytes (Fig. 8) and melanocytes. The latter contain many melanosomes, and the cytoplasm of the former has many iridophores or guanine crystals. This is in contrast to the findings of Spearman and Riley ('69), who report that iridocytes are absent in *Crocodylus niloticus*.

The epidermis in the ISO region (Fig. 9A) differs from the epidermis of non-ISO regions (Fig. 9B). Although the total thickness of the epidermis is equal in both regions, the ISO region has a thinner stratum corneum than the non-ISO region. Hence, the layer composed of the stratum germinativum and the suprabasal cells is more prominent in the ISO region, while the stratum corneum is reduced. Another difference between the ISO and non-ISO regions of the epidermis is that the stratum germinativum cells of the ISO region are more columnar in shape than those in other areas of the epidermis. Columnar germinal cells in reptilian sense organs have been noted in other studies (e.g., Maclean, '80).

#### DISCUSSION

The general morphology of the ISOs in *Crocodylus porosus* suggests that they are sensory structures of some form. The ISO is an opening in the stiff outer layer of the

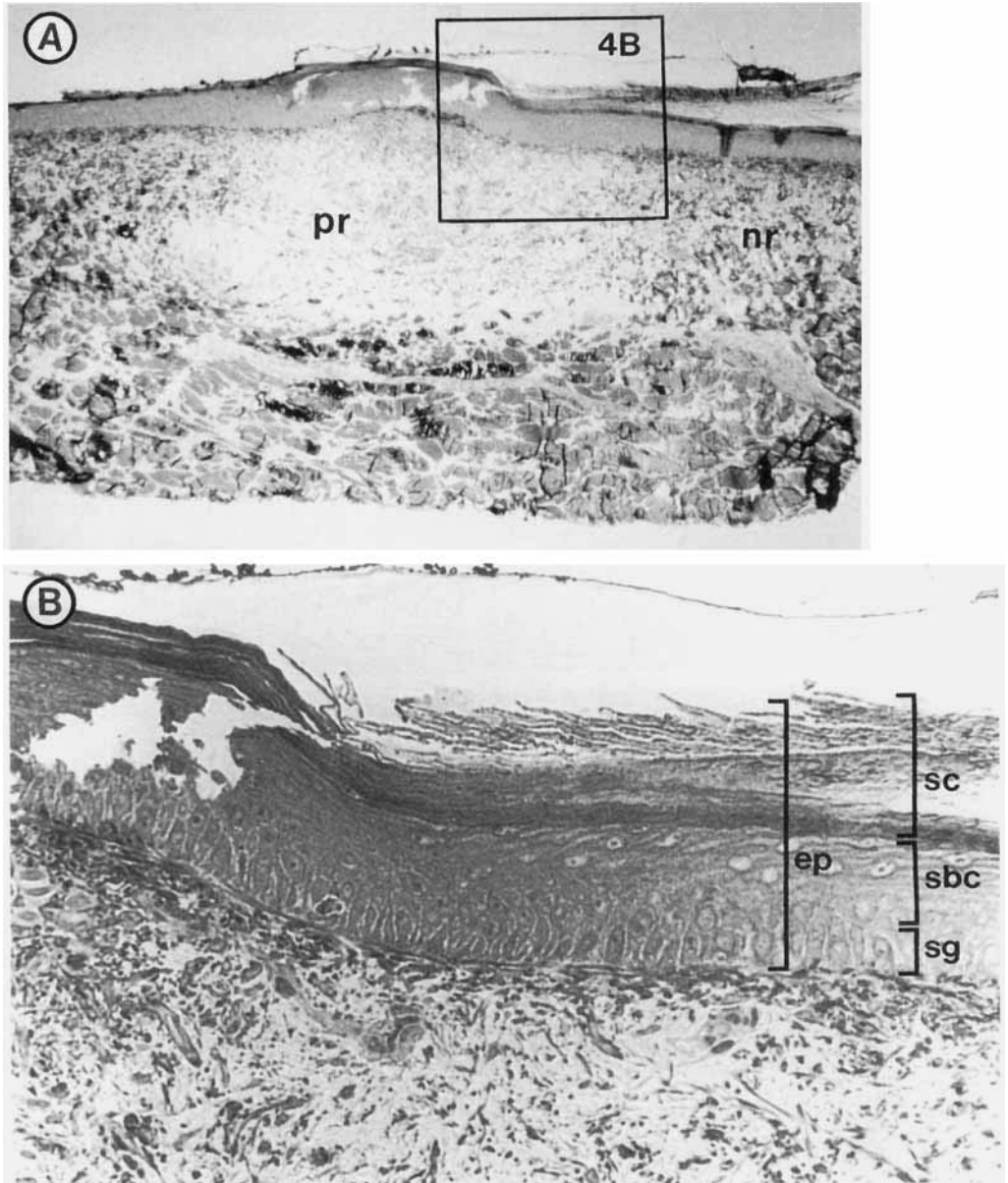


Fig. 4. *Crocodylus porosus*. Light micrographs of cross-sections an ISO (A) at  $\times 100$  magnification, showing the diffuse ISO region of the dermis (pr) and collagen-

rich non-ISO region (nr), and (B) at  $\times 470$  magnification, showing stratum germinativum (sg), suprabasal cells (sbc), and stratum corneum (sc) layers of the epidermis (ep).

stratum corneum through which a thinner, underlying layer of the epidermis is exposed. Immediately beneath this exposed region of the epidermis is a pocket in the dermis, perhaps fluid-filled. Nerve terminals are found in this pocket, immediately beneath the epidermis. The presence of nerve terminals is consistent with the hypothesis that the pits

are sensory organs, and we therefore favor the term *integumentary sense organs* (ISO) over other terms which have been used in the taxonomic literature (e.g., follicle glands, follicle pores, etc.).

The ISOs of *Crocodylus porosus* differ from known cranial touch papillae of *Caiman crocodilus* (von Düring, '73, '74) in several

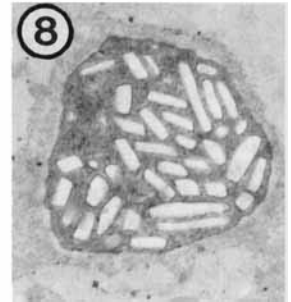
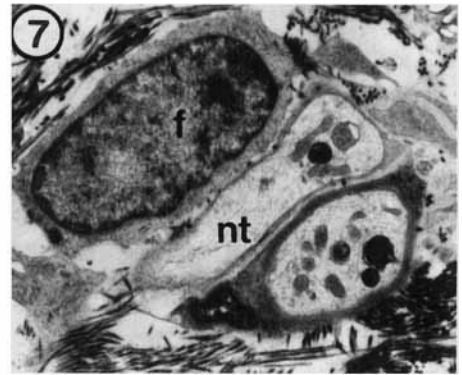
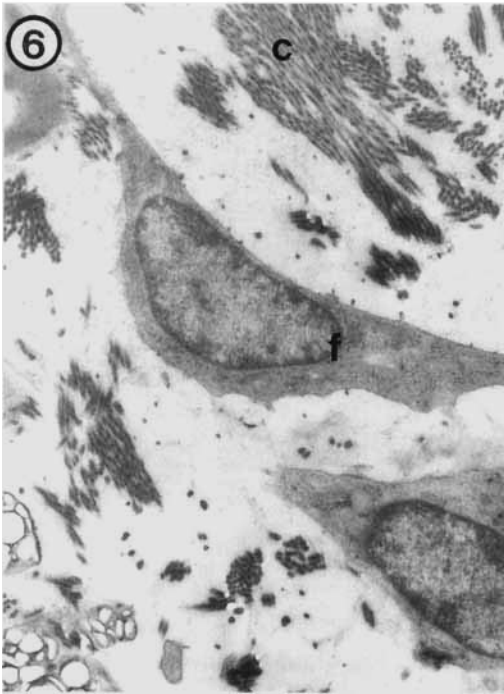
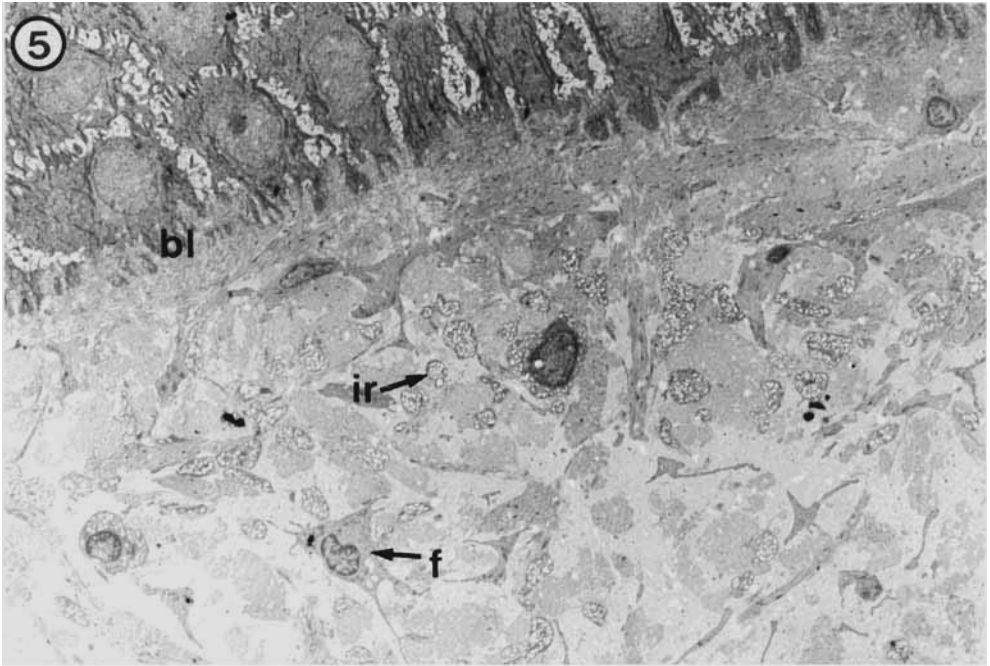


Fig. 5. *Crocodylus porosus*. TEM of the apex of ISO region of the dermis, showing high concentration of cells. bl, basal lamina of the stratum germinativum of the epidermis; f, fibroblast; ir, iridocyte.  $\times 1,800$ .

Fig. 6. *Crocodylus porosus*. TEM of fibroblasts from the ISO region of the dermis. c, collagen fibers; f, fibroblast.  $\times 5,600$ .

Fig. 7. *Crocodylus porosus*. TEM of nerve terminals (nt) supported by a fibroblast cell (f).  $\times 6,800$ .

Fig. 8. *Crocodylus porosus*. TEM of an iridocyte from the ISO region of the dermis.  $\times 10,300$ .

ways. 1) The ISOs are present on only the postcranial scales of crocodylids and gavialids, while the touch papillae are present on the cranial but not the postcranial scales of all crocodylians. 2) There is only one ISO on each postcranial scale (with occasional exceptions), while the number of touch papillae on the cranial scales, by contrast, is much more variable (4–16). 3) The ISOs are not concentrated in any one particular region of the skin, while touch papillae are most numerous on the scales surrounding the nares and the mouth and least numerous on the scales between the eyes. 4) Whereas each ISO is centered in the caudal third of the scale, the touch papillae are randomly distributed. 5) The ISOs are larger than the touch papillae (300  $\mu\text{m}$  diameter vs. 200  $\mu\text{m}$  diameter). 6) Although the outer surface of the ISOs is slightly convex, it is not raised to the degree described by von During ('73, '74) for cranial touch papillae, and the fluid in the diffuse pocket in the dermis of the ISO does not appear to be maintained under pressure as it is in touch papillae (von During, '74). 7) Although we identified nerve terminals in the ISOs, we did not observe all the receptor types described by von During in the touch papillae (intraepidermal nerve endings, Merkel cell neurite complex, lamellated receptors). Von During's touch papillae are unusual among reptilian sensory organs in having these structures which resemble mammalian Pacinian corpuscles. Although this apparent difference may reflect the fact that we used semithin sections and light microscopy for serial reconstruction and TEM only for fine detail, whereas von During ('73) concentrated on nerve terminals rather than on the entire structure and used TEM of thin sections for the entire serial reconstruction, it is more likely that this represents another difference between touch papillae and ISOs.

In spite of these differences, the overall morphology of the ISO is similar to that of the touch papilla. Both consist of nerve terminals contained in a diffuse, fluid-filled pocket in the outer surface of the dermis. This structural similarity may indicate functional similarity. However, it is also possible that the structural similarity has arisen as a result of homology and that they may have totally different functions.

Baden and Maderson ('70) have determined by x-ray diffraction that the stratum corneum of lepidosaur reptiles has an inner layer of alpha-keratin and an outer layer of beta-keratin, in contrast to that of *Alligator*,

which has a single layer of beta-keratin, with alpha-keratin only at the hinge regions of the scales. We observed, using SEM and TEM, what appeared to be two distinct layers in the stratum corneum of *Crocodylus*: a stiff outer layer which peeled off in flakes and a pitted and apparently more pliable inner layer. The outer layer tended to separate from the inner layer during sectioning, probably indicating noncontinuous beta-keratinization of unspecialized epidermis, as described by Menon et al. ('96).

The touch papillae have been described as mechanosensory on the basis of their structure (von During, '73, '74) and have been described as elevated relative to the surrounding integument as though under pressure from fluid inside (von During, '73). Figure 10 summarizes our observations of the morphology and ultrastructure of the ISO. The ISOs are not elevated relative to the surrounding integument. However, it is possible that the ISOs may also function as mechanoreceptors. We observed that the dermal region of the ISO has a high component of ground substance and little collagen and few cells. It is possible that these cells and fibers of the extracellular matrix (ECM) have been dispersed by a unique gel or fluid-like component of the ECM. This ground substance could be interpreted as an important element of the mechanoreception system which, when stimulated by external pressure, stimulates the nerve terminals near the epidermal-dermal junction.

However, there is no direct physiological evidence for mechanosensory function in ISOs, touch papillae, or any other sensory organ in the reptilian integument. To date only one study of integumentary mechanoreception in reptiles has been undertaken in which nerve action potentials were recorded as the integument was mechanically stimulated (Necker, '74). Because of the technical difficulty of recording electrical activity in the small efferent nerve fibers of individual mechanoreceptors, recordings are made from larger nerve fibers far downstream, so that it is impossible to attribute the observed response to the stimulation of any particular proposed sensory structure.

Although the ISOs may be mechanoreceptors, other functional interpretations are also possible. One such possibility is that the ISO is a chemosensory organ. The stratum corneum of the epidermis of the ISO region is thinner than that of the rest of the integument, with a surface that is pitted and possi-



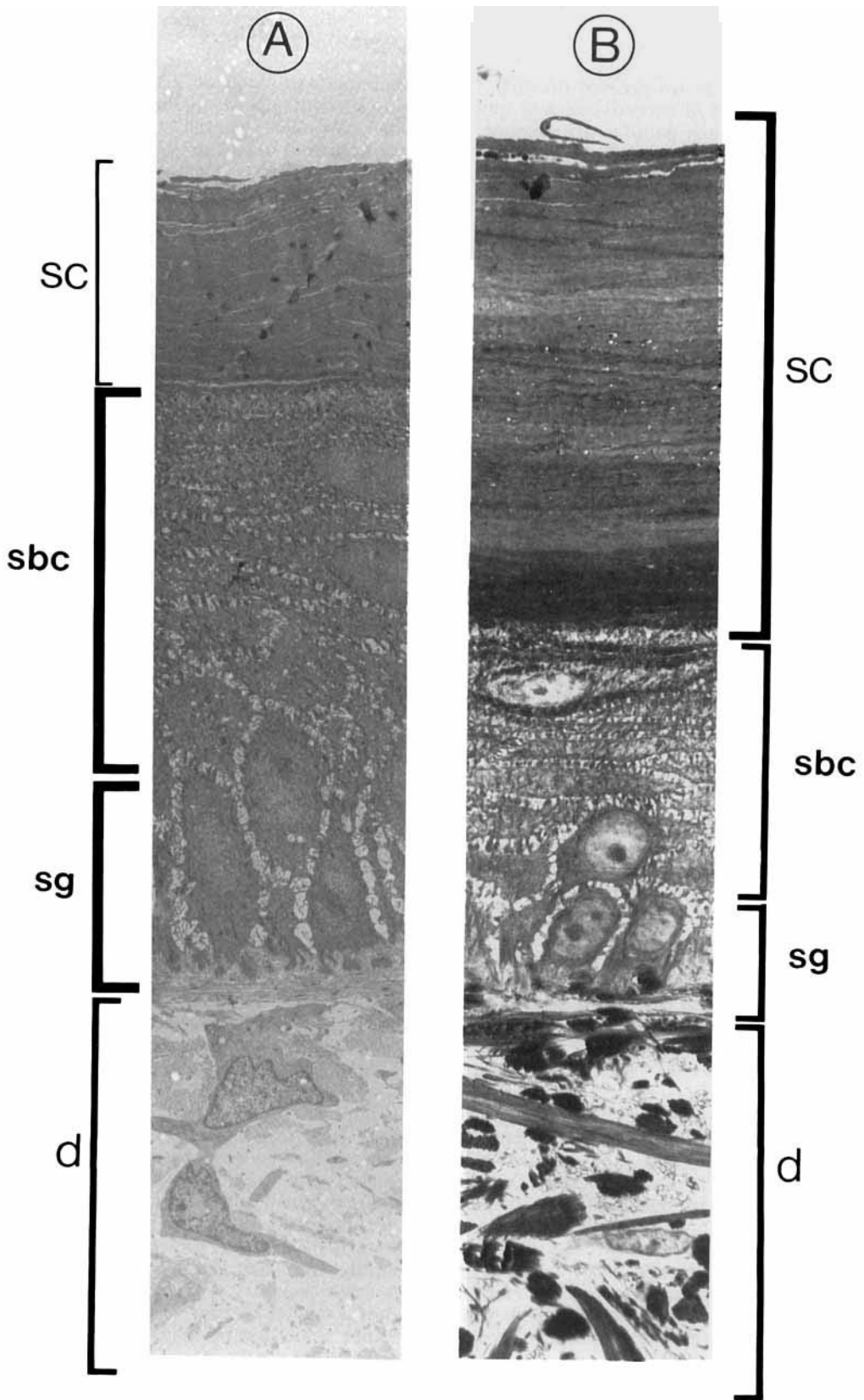


Figure 9



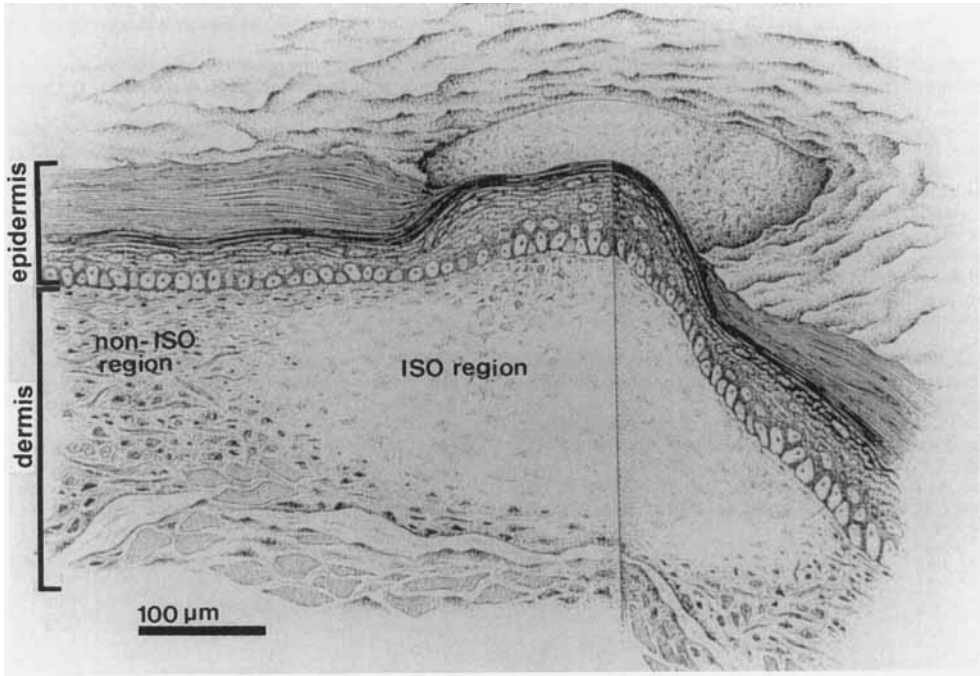


Fig. 10. *Crocodylus porosus*. Summary illustration of the integumentary sense organ.

bly porous rather than flaking off in flat sheets like the surface surrounding it. If the epidermis of the ISO region is indeed porous and allows the passage of fluid from the outside environment, then the diffuse, possibly fluid-filled area of the dermis in the ISO region could be interpreted as a sampling cell in which the nerve terminals of the ISO region are bathed in fluid from outside and are stimulated by the chemical characteristics of this fluid. It has been shown that crocodylids and alligatorids differ in their capacity for salinity discrimination (Jackson et al., in press), and one possible hypothesis concerning function is that the ISOs are involved in discrimination between hyper- and hypoosmotic salinities.

Morphological study of the ISOs reveals a structure which is potentially consistent with either a mechanosensory or a chemosensory function. If the function of the ISOs is to be determined, physiological study will be required. Such studies have the potential to

reveal whether the sensory function of the ISOs is mechanoreception or whether morphological differences between the ISOs and the cranial touch papillae reflect a functional difference between these organs.

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Fig. 9. *Crocodylus porosus*. TEM of epidermis from (A) the ISO region and (B) from another area of the same scale. d, dermis; sbc, suprabasal cells; SC, stratum corneum; sg, stratum germinativum.  $\times 2,600$ .

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