HISTOLOGICAL DESCRIPTION OF GENERATION GLANDS AND THEIR FUNCTIONAL RELATIONSHIP TO THE SHEDDING CYCLE IN CORDYLID LIZARDS

BY

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A THESIS PRESENTED TO THE UNIVERSITY OF STELLENBOSCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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MARCH 2002

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Abstract

GENERATION GLANDS AND SHEDDING CYCLES IN GIRDLED LIZARDS (SAURIA: CORDYLIDAE)

Specialized skin scales (generation glands) and undifferentiated skin scales were compared in three species of cordylid lizard, viz. *Cordylus cordylus*, *Pseudocordylus microlepidotus* and *P. capensis*. These skin derivatives were histologically examined and compared, using data existing in literature (Maderson, 1966; 1967; 1968a) with similar structures in gekkonids. Histological descriptions of cordylid skin structure were the same as those shown for gekkonids. Three types of generation glands were identified in cordylids (Van Wyk & Mouton, 1992), and these proved to be different to those existing in gekkonid species. These three types of generation glands were further compared regarding their ecdysis histology, which proved to be identical, thus, these glands differ only on gross morphological structure. Skin of all three species was also compared with regard to ecdysis, and while the histology of all three was identical, shedding activity was shown to differ in all three species. *P. microlepidotus* displayed dormancy in the germinative layer for much longer periods of the year than either of the other two species, while *P. capensis* displayed very little dormancy. *C. cordylus* displayed an intermediate pattern of ecdysis activity and dormancy. Autoradiography was performed on all species in both ecdysis activity and dormancy periods, to compare mitotic activity patterns in these periods. Generation gland activity appeared to correlate well with known testosterone peaks in the testicular cycle in all three species. Asynchrony between generation glands and skin scales was shown in all three species.

Uittreksel

GENERASIEKLIERE EN VERVELLINGSIKLUSSE IN GORDELAKKEDISSE (SAURIA: CORDYLIDAE)

Drie spesies gordelakkedisse, *Cordylus cordylus*, *Pseudocordylus microlepidotus* en *P. capensis*, se gespesialiseerde velskubbe (generasiekliere) en gewone velskubbe word vergelyk. Hierdie velskubbe word deur histologiese metodes geëksamineer en vergelyk, met gebruik van data wat reeds in die literatuur bestaan (Maderson, 1966; 1967; 1968a), met soortgelyke strukture in Gekkonidae. Histologiese beskrywing van Cordylidae velstruktuur was identies met dié van Gekkonidae. Drie tipes generasiekliere was geïdentifiseer in gordelakkedisse (van Wyk & Mouton, 1992), en hulle verskil gedeeltlik van naverwante strukture in Gekkonidae. Hierdie drie tipes generasiekliere was verder vergelyk met betrekking tot hulle vervellingsiklus histologie, wat identies was. Dus verskil hulle net met betrekking tot hulle vorm. Vel is ook vergelyk met dieselfde metodes. Alhoewel daar geen verskille was met betrekking tot hulle histology nie, was die tye van aktiwiteit van kiem-sellae beduidend verskillend in alle spesies. In *P. microlepidotus* het die kiem-sellaag russtadium baie langer geduur as in die ander twee spesies, terwyl *P. capensis* amper geen russtadium getoon het nie. *C. cordylus* het 'n intermediêre gedragspatroon vertoon tussen aktiewe en rustende fase met betrekking tot sy vervellingsiklus. Outoradiografie is gebruik op elke spesie, in beide aktiewe en rustende vervellingsfases, om verskille in mitotiese aktiwiteit te wys. Generasieklier aktiwiteit blyk te korreleer met pieke van testosteroonvlakke van die testikulêre siklus in al drie spesies. Asinkronie is aangetoon tussen generasiekliere en velskubbe in al drie spesies.

ACKNOWLEDGEMENTS

I would like to thank numerous people for their involvement and assistance throughout this project. I consider myself lucky to thank professors P.F.A. Maderson and J.H. Van Wyk for assistance with reptile skin histology, and particularly to thank Prof. Van Wyk for his enthusiasm in this field of study, and for numerous helpful ideas in how to write clearly.

I would like to thank Lanral Ruddock and Louise Visagie for their aid in the field collection of specimens, without their help I would have struggled to find many suitable sites. Dr Edmund Pool was invaluable for his explanations of the autoradiographic technique and in vitro cell cultures.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF COMMONLY USED ABBREVIATIONS

SG	stratum germinativum
β_I	β-layer of inner epidermal generation
αi	α-layer of inner epidermal generation
$p\beta_I$	presumptive cells of β-layer of inner epidermal generation
pαi	presumptive cells of α-layer of inner epidermal generation
βo	β-layer of outer epidermal generation
αo	α-layer of outer epidermal generation
lto	lacunar tissue layer of outer epidermal generation
clo	clear layer of outer epidermal generation
Obi	oberhautchen of inner epidermal generation
Obo	oberhautchen of outer epidermal generation
OG	Outer Generation
IG	Inner Generation
m	mantle
GG	Generation Gland
FG	Femoral Gland
SS	Skin Scale

ONE

LITERATURE REVIEW

1 INTRODUCTION

1.1. BROAD INTRODUCTION AND OBJECTIVES OF REVIEW

Skin is the largest organ in the body of all vertebrates, if one takes the definition of skin as "a group of similar cells grouped together to perform a common function". It covers and protects the organism against a multiplicity of threats (dehydration, physical trauma, invasion by pathogenic organisms, etc.). Dermatologists have studied mammalian skin in detail, with whole textbooks dedicated to mammalian epidermal morphology and physiology (Spearman, 1973). Reptilian skin, in contrast, is not as well investigated (Maderson, 1964).

Skin and skin glands have been investigated in several reptilian species (Chiu & Lynn, 1970; 1971; 1972; Chiu et al., 1983; Irish et al., 1988; Landmann, 1979; Maderson & Chiu, 1975; Maderson & Licht, 1967; Maderson et al., 1972; 1998; Mittal & Singh, 1987a; 1987b), most notably the geckoes (Chiu et al., 1986; Chiu & Maderson, 1980; Flaxman & Maderson, 1973; Kluge, 1983; Maderson, 1963; 1967; 1968; 1972; Maderson & Chiu, 1981; Menchel & Maderson, 1975; Zucker & Maderson, 1980). Research directions have included descriptive morphology, histology, electron microscopy and hormonal interaction. There are few reviews to date pertaining to skin (Maderson, 1964; 1985, 1986) and skin glands (Cole, 1966; Maderson, 1970; Maderson & Chiu, 1970) in reptiles. This review will build on the foundations laid by these researchers.

Skin morphology and dynamics, and the various types of generation glands, are explained in generalized terms. This should enable the reader to better understand the terminology and significance of subsequent chapters in this thesis.

1.2. BRIEF OVERVIEW OF SKIN AND SKIN SHEDDING IN REPTILES

1.2.1. Generalized Skin Histology

The clearest description of a "generalized" squamate epidermis is provided by Maderson (1965, 1966, 1998). Lepidosaurian skin is exfoliated in discrete epidermal generations, as opposed to the continuous exfoliation of skin cells in mammals (Flaxman et al., 1968; Maderson, 1966; Roth & Maderson, 1968). Each mature, complete generation is composed of six layers, each of a different cell type (Figure 1a). The new generation underneath is incomplete as the mature generation sloughs off (Figure 1b).

The innermost layer of squamate skin is the *stratum germinativum* (SG), which is the germ layer that produces the cell types that make up an epidermal generation. The cells of this layer are basophilic, and vary in shape from columnar to cuboidal to extremely flattened, depending on which stage of epidermal renewal is present.

Immediately adjacent to the SG is the *clear layer*, which is composed of living cells. This layer forms part of the shedding complex (zip-fastener mechanism) (Maderson, 1966). Next is the *lacunar tissue layer*, which is also composed of living cells. This is the only cell type that has nuclei in the mature tissue, as the other cells types nuclei are pycnotic. They are characteristically swollen and have an affinity for aniline blue (Maderson & Licht, 1967). The K-*layer* (loose, keratinized tissue, which stains pink with eosin) is situated above the lacunar tissue layer. It is very compact tissue of extremely flattened cells, which is considered to be the primary barrier to cutaneous water loss (Zucker & Maderson, 1980). The *mesos layer* (consisting of only refractile strands under light microscopy) is situated above the α-*layer* and underlies the 2-*layer*, which is a compact, keratinized, chromophobic tissue. Cells of the β-layer form a syncitium, which protects against physical damage, maintains the shape of the scale and protects the important α-*layer*. They do not contain lipids, and so do not contribute to the barrier against water loss (Lillywhite & Maderson, 1982). The last layer is the *oberhautchen*, which is the outer surface of the 2-*layer*. This is a single cell layer thick, and often bears micro-ornamentation in the form of spikes and hooks (Harvey & Gutberlet, 1995;

Maderson, 1998). The "zip-fastener" mechanism is thought to be the inter-locking of these spines with the clear layer of the outer generation (Maderson, 1966).

The epidermis of Lepidosaurian scales is composed of these basic layers. Modified scales, such as generation glands, differ in gross morphology and histology from normal unspecialized scales. Glandular material of generation glands is produced in a holometabolic manner, either by an additional layer of a specialized cell-type, or an existing layer that is altered (Maderson, 1972). Production of new layers of epidermis is part of the process of skin shedding.

1.2.2 Skin shedding

Comprehension of the skin shedding process is invaluable in understanding the dynamics of glandular structures. All scales of the Lepidosaurian epidermis are shed at the same time, and as glandular scales are part of the epidermis, they shed at the same time. New glandular material is exposed as the old epidermal generation is shed (Maderson, 1967).

There are six standard stages in generalized squamate skin shedding, according to the outline provided by Maderson (1966, 1985) and Maderson et al. (1998). There is a period of rest before skin shedding, when the SG is not producing new epidermal cell types. During this time, the epidermal generation consists of a α-layer, mesos layer, β-layer and oberhautchen.

When active shedding proliferation begins, the SG begins to produce new cells: the lacunar tissue layer and clear layers are produced, completing the outer epidermal generation. Proliferation continues to form the α-layer, mesos layer, β-layer and oberhautchen of the new epidermal generation. Once these are completed, the complete outer epidermal generation is shed (Figure 1b).

In more detail, the rest phase can be divided into three arbitrary stages: immediately post shed stage (IPS), perfect rest stage (PRS) and pre-shed stage (PSP). IPS is characterized by an active SG, with an incomplete skin generation above it, consisting of an α-layer, mesos layer, β-layer and oberhautchen. This incomplete epidermal generation has many presumptive and few mature K-layer cells, as the K-layer is incomplete post-shedding. The mesos and 2-layers are mature, with the syncitium of 2-layer and oberhautchen complete. PRS differs in that the SG is quiescent, and there are only mature K-layer cells. This condition is

maintained until initiation of PSP. The pre-shed stage is characterized by an active SG and cells that will become the clear and lacunar layers.

Stage two is characterized by the production of oberhautchen cells, a single layer of cells with spinules that will interdigitate with the over-lying clear layer of the outer generation to form the 'zip-fastener' mechanism. Above the new oberhautchen lies the outer epidermal generation. The lacunar tissue layer of this mature generation consists of several layers of swollen cells that stain well with blue stains, while the clear layer appears as a line of cells that stain slightly darker with haematoxylin.

Stage three is characterized by the production of several layers of cells that will form the β-layer. These are flattened polygonal cells that are slightly eosinophilic. These cells are produced both in stage three and into early stage four. These β-layer cells are presumptive, and form a much thicker β-layer than the mature β-layer found in the outer epidermal generation.

Stage four is characterized by the cessation of β-layer cell production and the onset of production of mesos layer cells. These cells are extremely flattened and are basophilic.

Stage five is characterized by the formation of the β-layer/oberhautchen syncitium as these layers mature in the inner generation (losing cell membranes and nuclei becoming pycnotic and disappearing), and the initiation of presumptive α-layer cell production. The mesos cells become even more flattened and stain much lighter than the α-layer cells. The clear layer of the outer epidermal generation matures to become a line of keratinized material, which may fragment along vertical membranes.

Stage six is characterized by the skin shedding. Presumptive α-layer cells continue to be produced and are added to the α-layer.

The shedding cycle has been reported to be regulated by hormones. Although much data has been collected on this topic (Chiu & Lynn, 1970; 1971; 1972; Chiu et al., 1970; Flaxman et al., 1968; Maderson, 1985; Maderson & Licht, 1968), the stimulation and control of shedding is not well understood (Flaxman, 1972; Maderson, 1985). In snakes, the presence of thyroid hormones has been shown to be an inhibitor of cellular proliferation and differentiation in the process of

skin shedding, maintaining the perfect resting phase. This is indicated by elimination of the resting phase in snakes that have been thyroidectomized (Chiu & Lynn, 1971; 1972; Maderson & Chiu, 1975). In lizards, however, the opposite is true, with thyroidectomy causing extension of the rest phase (Chiu & Lynn, 1970). Other hormones influencing the shedding cycle include ACTH (inhibits shedding) and prolactin (increases shedding frequency) (Maderson & Licht, 1967; Chiu & Lynn, 1970).

Reptilian epidermal glands are very sensitive to androgens, which can cause these glands to develop on previously unspecialized skin scales. These hormones also cause skin shedding to increase in frequency. The action of these hormones is unclear, although it is speculated that these hormones stimulate general metabolism in the body (Maderson & Chiu, 1981).

As previously stated, the skin is shed in generations, with the entire old generation being shed at once. Thus, all the scales are in synchrony (Chiu et al., 1975). The concept of synchrony is important when the process of shedding is to be discussed with regard to glandular activity in later chapters.

1.3. HOLOCRINE EPIDERMAL GLANDS IN REPTILES: AN OUTLINE

Reptiles are renowned for the scarcity of glandular structures in their epidermis. This is possibly because reptiles were the first vertebrates to become fully adapted to a terrestrial habitat, and survival favoured individuals in which skin developed in such a way as to minimize fluid loss (Spearman, 1973). Those epidermal glands present in lizards are represented not only by the large sebaceous types located on the femoral or pre-cloacal areas (Cole, 1966; Quay, 1986), but also by a selection of other intragenerational holocrine epidermal glands (Maderson, 1970; Quay, 1986; van Wyk & Mouton, 1992). Therefore, holocrine glands in reptiles may be divided into two basic categories. One is the tubulo-follicular "femoral" or "pre-cloacal" glands, the other is the generation glands, which include both the escutcheon scale and beta-glands of gekkonids and the generation glands of cordylids. The callous glands of agamids (Dujsebayeva, 1998) are subjects of current controversy, with differences in opinion where these may be classified (van Wyk, pers. comm.). For the purposes of this review, I will categorize them with the generation glands, based on callous glands not being tubulo-follicular like the

femoral glands. All of these gland types vary in morphology, but produce glandular material in a holocrine manner.

1.4 EPIDERMAL GLANDS: GENERAL DIVERSITY AND DISTRIBUTION IN LIZARDS

1.4.1. Femoral glands

These glands are easily discerned by their tubulo-follicular appearance (Cole, 1966; Quay, 1986) and derive their name from their location on the lizard's body, viz. in a single, unbroken row on the postero-ventral margin of the thigh. Femoral glands have been used for taxonomic purposes for many years (Linnaeus, 1758; Lang, 1991). Cole (1966) presents the most recent and comprehensive review on the topic.

In generalized terms, the femoral gland consists of a gland body below the dermis, extending from the pore on the postero-ventral margin across the plane of the femur, in the region below the generation glands. The gland body is separated from surrounding muscles of the femur by a membrane, or envelope. This gland body extrudes cells in a holocrine fashion along a femoral gland canal passing through the dermis, terminating in a femoral gland pore. Externally this is visible as a femoral gland scale with a pore in the middle of the outer scale surface (OSS). The pore is filled with a secretion plug, which may consist of several to many secretion rods that are easily extruded with mechanical pressure (Ruddock, 2000).

The gland body shows much variation in size and complexity, varying between species and sex. Interspecific differences occur because of many factors, including varying body size and sexual dimorphism. Sexual dimorphism is usually expressed by males possessing much larger femoral glands than females. Individual glands on one individual may vary according to their position. Glands found in the centre of the row are often more complex, i.e., more follicles per gland, and those proximal to the knee and groin are more simple, with much fewer follicles (van Wyk, 1990).

Material produced in the follicles travels down the pore canal to the pore, where it is ultimately excreted. Femoral glands produce a copious and continuous holocrine secretion, because the cells' production and subsequent differentiation is

independent of the normal body epidermis (Fergusson et al., 1985). This secretion is known to contain protein and volatile lipids (Alberts, 1990; Alberts et al., 1992b; Fergusson et al., 1985). Each follicle produces a single secretion rod. Large species such as *Cordylus giganteus* have as many as eight secretion rods (Ruddock, pers. comm.), whereas smaller species like *C. cordylus* and *Pseudocordylus capensis* have fewer rods, often between two and three rods per pore (personal observation).

Femoral glands occur in many lizard families (van Wyk & Mouton, 1992). Within families, diversity may occur. This diversity is often due to sexual dimorphism, with males possessing femoral glands while females have reduced femoral glands, as is the case in *Gekko gecko* (Maderson, 1985).

1.4.2 Generation glands

Grant (1931) first documented generation glands on members of the gekkonid genus *Sphaerodactylus*. Several researchers (Noble, 1940; Noble & Klingel, 1932) preliminarily examined the microscopic structure of these scales. In 1956, Taylor & Leonard conducted the first detailed microscopic investigation of these glands' structures. This study was expanded by the involvement of additional researchers (Maderson, 1967; 1968a; 1968b; Chiu & Maderson, 1975). Cordylid generation glands were investigated microscopically by van Wyk & Mouton (1992).

Generation glands are modified skin scales, where the OSS is modified for holocrine, glandular material production. Various situations are known to occur in three families, Gekkonidae, Cordylidae and Agamidae. Generation glands all produce new glandular material each time the scale goes through the shedding cycle. This glandular material is exposed as the mature generation is shed, resulting in the name 'generation glands'. As such, they are all collectively placed in the category 'generation glands'. They are sub-divided in this review according to which particular skin layer produces the glandular material. Escutcheon scales produce glandular material in a modified oberhautchen layer, a situation found in Gekkonidae (Maderson, 1967). β-glands produce glandular material in a modified β-layer, a situation occurring in both Gekkonidae (Maderson, 1968a) and Cordylidae (van Wyk & Mouton, 1992; van Wyk, 1997a).

They are potentially present in several other families, as observations have been made in other taxa, although no histological detail is yet available. Researchers postulate that they may be present in most gerrhosaurids (van Wyk, pers. comm.), one iguanid (Maderson, 1970) and one scincid species (O'Shea, 1991; Russell, 1997).

Individual glandular scales usually occur in patches, each producing its own glandular material. In all families where generation glands are found, they are usually located on ventral surfaces such as the ventral femoral region, abdomen or cloacal area. There are some exceptions at the species level, such as *Pseudocordylus microlepidotus*, which has dorsal generation glands in addition to those glands on the ventral regions, and *Cordylus giganteus*, which possesses generation glands on the forelimb regions in addition to those under the thigh (van Wyk & Mouton, 1992).

1.4.2.1 Escutcheon scales

Glandular scales are regarded as normal scales that have modified skin layers producing the glandular material. The outer scale surface is always distinctly concave with the deepest portion located at the posterior, distal portion of the scale. It is in this concave portion where the specialized epidermal material is seen in gekkonids (Maderson, 1968a; 1968b). The generation glands of the gekkonid lizards are situated on the posterior abdominal or ventral femoral regions (Maderson, 1967; 1968a; 1968b; Maderson & Chiu, 1970; Taylor & Leonard, 1956) and around pre-cloacal organs in *Lygodactylus* (Maderson, 1970).

In escutcheon scales, glandular material is formed by the clear layer. This material is deposited on the oberhautchen of the new generation as the old generation is shed, and is only exposed immediately after shedding. Species possessing escutcheon scales shed frequently, therefore this material is released, or exposed, quite regularly. Escutcheon scales are observed in the sphaerodactyline gekkonids *Gonatodes*, *Lepidoblepharis* and *Sphaerodactylus* (Maderson, 1967; 1970; Menschel & Maderson, 1975; Taylor & Leonard, 1956).

1.4.2.2 β-glands

Glandular material of the "β-gland" is produced between the β-layer and the oberhautchen, and is formed either from enlarged oberhautchen cells or from an additional layer of cells between the oberhautchen and the β-layer (Maderson, 1968b). The result is that the glandular material becomes exposed as the old generation is shed. The oberhautchen cells become elongated and obliquely orientated, with rounded nuclei in a cytoplasm that is initially granular, and later acidophilic and homogenous. Due to this conversion, the oberhautchen is non-continuous over the middle of the scale. This is observed in no other part of the epidermis (Maderson, 1970). This type of gland is found in the diplodactyline gekkonids, e.g. *Phyllurus milii*, *P. platurus* and some species of *Diplodactylus,* and in the gekkonine *Teratoscincus* (Kluge, 1983). β-glands are present in the iguanid *Leiocephalus* (Alexander & Maderson, 1972; Maderson, 1970).

To date there is limited data on the histology and gross morphology of cordylid generation glands. van Wyk & Mouton (1992) describe two main generation gland types in cordylids. These are categorized as either pit glands or stacked glands, according to the depth at which the SG is situated. Pit glands have a SG that is lower than that of surrounding undifferentiated scales, thus producing the glandular material in a pit. Glandular material is stacked higher than surrounding scales in the stacked glands, because the SG is at the same level as that in surrounding scales. Stacked glands are subdivided into single-layer stacked glands and multi-layer stacked glands, while pit glands are exclusively multi-layered. Information to date is simply that glandular material is produced by the β-layer, but no information is provided about the other layers in each generation. As the family Cordylidae is under revision, the glands cannot be positively linked to specific genera. Pit glands are only found in *Pseudocordylus microlepidotus* and *P. melanotus*, while single-layer stacked glands occur in *Platysaurus* species and *Pseudocordylus capensis*. Multi-layer stacked glands are found in *Cordylus* species (van Wyk & Mouton, 1992; van Wyk, 1997a).

1.4.2.3. Callous scales

Occurring in the family Agamidae, these glands are found on the ventral abdominal and pre-anal regions. They are observed to be in non-overlapping parallel rows, 1 – 7 rows deep, and appear swollen under close observation.

Currently the subject of taxonomic controversy, these glands have only recently been histologically examined (Dujsebayeva, 1998). They were initially described by Boulenger (1885) as 'callous pore-like swellings of the preanal scales'. Harris (1963) described them as preanal callous pads, whereby each gland was a modified scale with a deep, stratified epithelium and a thick outer layer of dead compressed cells. Moody (1980) redefined them as precloacal glands, because the external opening in lizards is a cloaca, not an anus. The histological description by Dujsebayeva is as follows: a SG composed of round or cuboidal cells, with 7-20 layers of living cells above it. These cells have round or ovoid nuclei and a granular cytoplasm. Above this is an upper layer of keratinized cells that form a deep stratified epithelium. Cells in this layer have thick eosinophilic membranes, pycnotic nuclei and a basophilic cytoplasm. The outermost layer is composed of very strong flattened keratinized cells. A histochemical analysis showed that the callous secretion (presumably the glandular material) had a weak reaction for neutral mucopolysaccharides similar to that of α-layer cells. On these grounds, the α-layer was determined to be the secreting layer. No other skin layers were named apart from saying that the β-layer and oberhautchen appeared to be missing, thus indicating an active function. Dermal papillae rich in blood vessels penetrate into the epidermis in these glands and the epidermal portion invaginate into the dermis in long folds (Dujsebayeva, 1998).

Since no single centrally located pore is present and a generation-like build-up of glandular generations is noticed, it is speculated that these glands may represent a gland-type that has characteristics of both generation glands and femoral glands.

1.5. FUNCTION OF EPIDERMAL GLANDS

Cole (1966) speculated about the function of femoral glands, coming to no specific conclusions. Investigation of the components of femoral gland secretions have shown that it is composed of circa 80 % protein and 20 % volatile lipid (Alberts,

1990; Fergusson et al., 1985). Lipids have potential to act as pheromones. The capacity for lipids to form structural and geometric isomers, as well as the interchangeability of lipid functional groups, render a high degree of molecular diversity (Alberts et al., 1992b; Weldon et al., 1990). This increases the potential information content if the function of the gland is pheromonal (Alberts et al., 1992a; 1992b; Duvall, 1979). Further supporting this hypothesis, lipids have been shown to act as semiochemicals, aiding in sex discrimination in reptiles (Cooper et al., 1996; Cooper & Garstka, 1987; Mason & Gutzke, 1990).

Proteins within secretions are not very volatile and may remain in the environment for longer periods than volatile lipids. Proteins in the glandular secretion show more interspecific than intraspecific variation, emphasizing the variation of chemical information to be derived from glandular deposits. Investigations into the individual differences within proteins showed that they could act as individual recognition markers, as individual differences remain stable throughout the year (Alberts, 1990; Alberts & Werner, 1993; Alberts et al., 1993).

The location of epidermal glands along areas of the body that are in regular contact with the substrate is ideal for encouraging distribution of exudate by abrasion. Thus, areas where the lizard spends most of its time would contain more glandular deposits than others. It is therefore likely, especially among sit-and-wait foraging, rupiculous lizards like cordylids, that these chemicals could be used for territorial marking and sexual recognition (Cooper et al., 1996).

Sexual dimorphism in gland morphology could further explain the chemo-communicative properties of epidermal glands in male behaviour (Cole, 1966; Dujsebayeva et al., 1998; Mouton & Van Wyk, 1993). In those species where femoral glands occur in both sexes, the pores are generally larger in males. In some species, only males possess femoral glands, and in other species where both sexes possess femoral glands, some females may possess femoral glands and others may not (Cole, 1966; Cordes et al., 1995).

1.6 HORMONAL INFLUENCE AND SEASONALITY WITHIN EPIDERMAL GLANDS

Epidermal glands are part of the reptilian skin, and as such are affected by the normal hormones that regulate the skin's activity. They are also used as

secondary sexual characteristics in many taxonomic descriptions (FitzSimons, 1943; Lang, 1991; Loveridge, 1944). This implies that they are influenced by sex hormones. The majority of experiments to date relate to the effects of sex hormones on epidermal gland activity (Alberts et al., 1992a; Abell, 1998; Chiu et al., 1970; Maderson & Chiu, 1981).

Femoral glands are reported to be more active during the breeding season (Cole, 1966) and femoral gland activity has been positively correlated with the sexual cycle of the testes in *Sceloporus undulatus undulatus* (Altland, 1941). More recent research has shown that androgens have a positive influence on the activity of femoral glands. Exogenous testosterone stimulates femoral gland secretion and causes an increase in femoral pore diameter in *Amphibolurus ornatus* (Fergusson et al., 1985) and *Sceloporus virgatus* (Abell, 1998). *Cordylus polyzonus* shows an increase in femoral gland size that correlates with an increase in size of the testis and seminiferous tubules during the reproductive cycle (van Wyk, 1990). In *Iguana iguana*, a positive correlation was found between circulating testosterone levels and the mass of the femoral gland secretion (Alberts et al., 1992a). Estrogens are postulated to have an inhibitory action on femoral glands (Alberts et al., 1992a).

Generation glands are similarly affected by androgens. Exogenous testosterone causes the development of generation glands in females of species where they normally do not possess glands, such as *Hemidactylus flaviviridis* (Chauhan, 1987), *Gekko gecko* L. (Chiu et al., 1970), *Hemidactylus bowringii* (Chiu et al., 1975) and several other species (Maderson & Chiu, 1981, Chauhan & Chauhan, 1985). Removal of androgens (hypophysectomy) causes regression of generation glands in male lizards. This regression can be reversed by application of exogenous androgens (Maderson & Chiu, 1981).

The number and development of generation glands in gekkonid species have been investigated in annual cycles, and together with other studies that measured testosterone cycles, provided circumstantial evidence that the variation in presence and development of generation glands can be linked to the circulating titer of endogenous androgen, which will change with the seasons. Generation glands reduce in number and size in the non-breeding season (Chiu & Maderson, 1975; Chiu et al., 1975).

1.7 EVOLUTION OF EPIDERMAL/GENERATION GLANDS

Many possible paths of evolution from unspecialized epidermal scales to functioning glandular scales or femoral glands have been postulated (Kluge, 1983). The most parsimonious solution is that femoral glands are derived from generation glands of some sort, which in turn are derived from unspecialized scales. Thus, a femoral gland is likely to have evolved from a generation gland that has become asynchronous with surrounding skin scales (Maderson, 1970; Maderson & Chiu, 1970). Maderson further suggests that, due to the abundance of families possessing epidermal glands, any species possessing no glands at all would indicate that glandular structures have been lost during evolution, and that this is a derived state (Maderson, 1970).

1.8. AIMS AND OBJECTIVES OF THE THESIS

This thesis presents a study of the three types of generation glands in cordylids. The objective of chapter two is to describe each type in close histological detail. Histological description could yield important information regarding chemo-communicative function, and would answer some questions posed by previous researchers (van Wyk & Mouton, 1992).

Chapter three's objectives are to describe the cytological changes in the epidermis of cordylids during the various stages of epidermal renewal, both in the normal, unspecialized skin, and the generation glands; and to ascertain any seasonal aspects of the shedding cycle of unspecialized skin or generation glands in these species.

Chapter four extends the objective of finding seasonal activity of generation glands by using autoradiographic techniques and scintillation counts. The individual primary objectives of chapter four are to use these techniques to determine (1) any species variation in epidermal renewal rate as shown by differences in the mitotic activity in the SG of the unspecialized skin scales and generation glands; (2) any epidermal renewal rate variation between unspecialized skin scales, and specialized generation glands; (3) any seasonal variation in epidermal renewal rates of the SG in activity on unspecialized skin scales of representative species displaying the different generation gland types, and seasonal variations between the gland types themselves. A secondary objective of this experiment was to show, using radioactive labelling and relative mitotic activity, the feature of asynchrony between generation glands and unspecialized skin scales in cordylid lizards

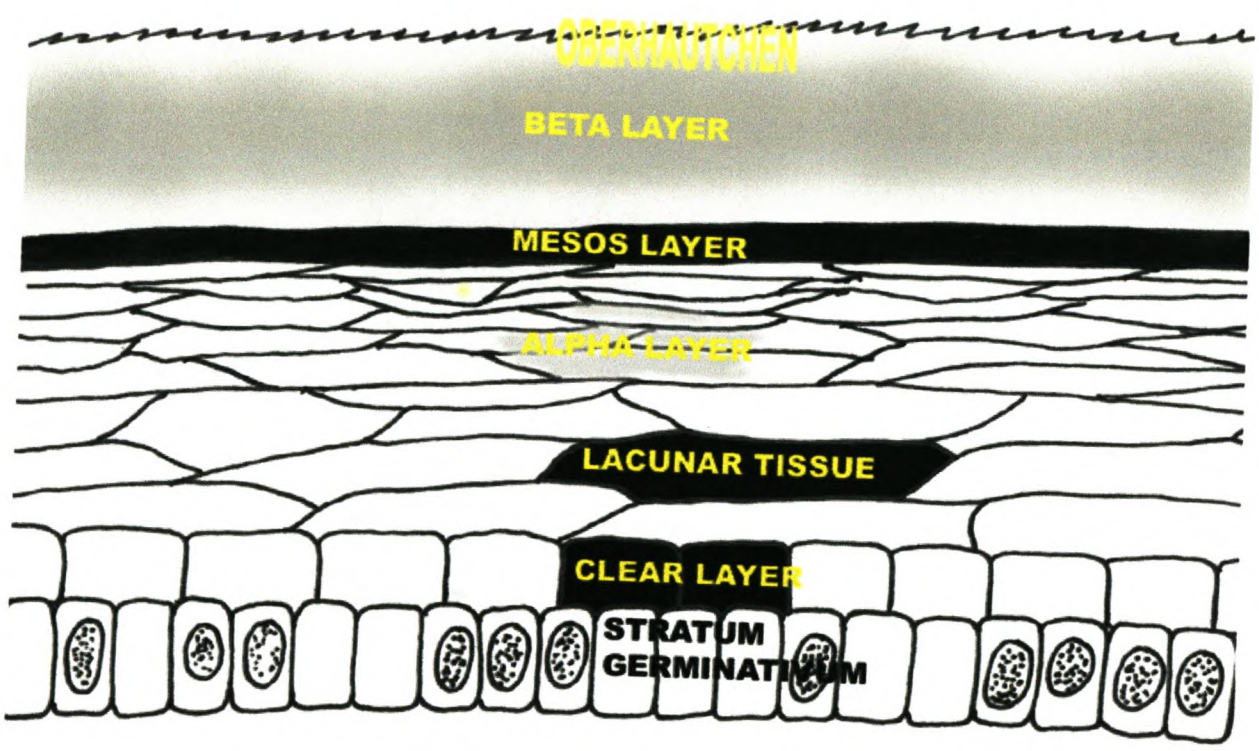


Figure 1a: All the possible layers in unspecialized squamate epidermis.

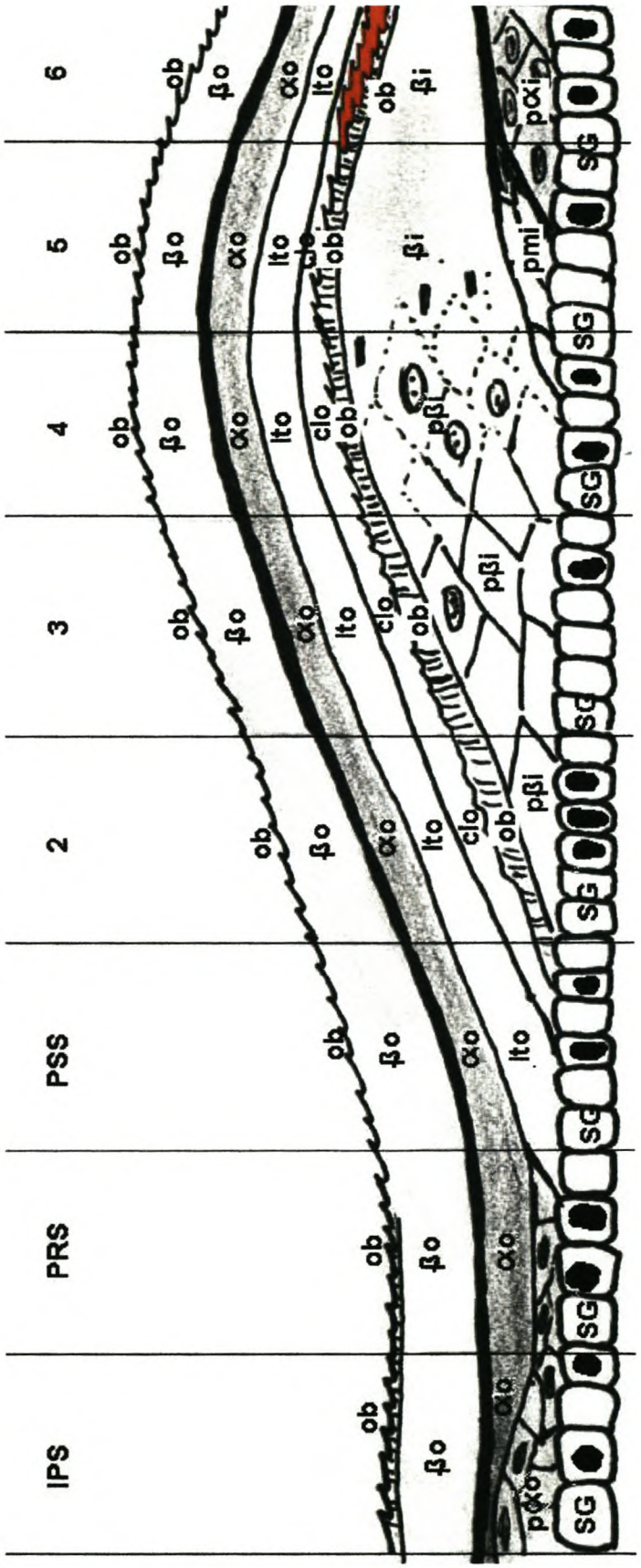


Figure 1b: All the stages of skin shedding of the generalized Lepidosaurian epidermis (For all figures, see: List of Commonly Used Abbreviations, page XV)

TWO

THE HISTOLOGICAL DESCRIPTION OF UNSPECIALIZED CORDYLID SKIN AND ASSOCIATED GENERATION GLANDS

2.1. INTRODUCTION

Generation glands are specialized epidermal scales, usually ventrally situated, on the abdomen or thigh of two lizard families (Gekkonidae and Cordylidae)(Maderson, 1970; van Wyk & Mouton, 1992). Glandular secretion is produced in a holocrine manner, and is hypothesized to have a chemo-communicative function (Cooper et al., 1996). Generation glands have been examined in Gekkonidae, showing there to be two types of generation gland in this family (Maderson, 1967, 1968a, 1970). Three different gland types are found in Cordylidae, (van Wyk & Mouton, 1992).

Histological description of generation glands in gekkonids identified β-glands and escutcheon scales (Taylor & Leonard, 1956; Maderson, 1970; 1972). Both are normal skin scales, modified to produce glandular material by either the addition of a new layer of cells in the epidermal generation (β-glands have a new layer between the β-layer and the oberhautchen), or the modification of an existing layer (escutcheon scales have a modified clear layer) to produce glandular material (Maderson, 1970). Cordylidae have been shown to possess generation glands that differ from any previously described glands (van Wyk & Mouton, 1992). Femoral glands are located in a row on the postero-ventral margin of the thigh. The generation glands are located in a patch immediately adjacent to these. These generation glands do not shed mature epidermal generations, resulting in a build-up, or 'stack' of layers, of varying heights. Not only do the generation glands in Cordylidae differ morphologically from those described in gekkonids, the three gland types differ from each other. The cordylid generation glands have been described as either single-layer stacked glands, multiple-layer stacked glands or multiple-layer pit glands. The marked difference between the stacked and pit glands is that the stratum germinativum (SG) of the stacked gland is at the same level as that of the surrounding unspecialized skin scales (resulting in a stack that protrudes above the

level of surrounding scales), whereas the pit gland's SG lies below the level of that of the surrounding scales' SG (build-up of layers does not protrude above the surrounding skin) (van Wyk & Mouton, 1992).

Previous description of cordylid generation glands (van Wyk & Mouton, 1992) indicated the presence of the stratum germinativum and β-layer, but made no mention of the other layers of the typical Lepidosaurian epidermal generation. The authors stated a need for more detailed investigation of cordylid generation glands to determine to which gekkonid generation gland type the cordylid generation gland most closely corresponds.

The goals of this chapter are to describe and compare the gross morphology and detailed histology of unspecialized skin and three generation gland types in cordylid lizards, previously introduced by van Wyk & Mouton (1992). Histological description could yield important information regarding both chemo-communicative function and phylogeny.

2.2. MATERIALS AND METHODS

To describe the histology of three types of generation glands, biopsies were taken from *Pseudocordylus capensis* (single-layer stacked gland), *Cordylus cordylus* (multi-layer stacked gland) and *P. microlepidotus* (multi-layer pit gland). Lizards from each species were taken from existing museum material from the J. Ellerman Museum of the Zoology Department at the University of Stellenbosch. Additional specimens were collected by noosing, later sacrificed, and skin samples were taken from the left thigh. The skin sample was taken from the area on the glandular patch where the glands were most closely packed together, in order to section the maximum number of glands in one series.

Samples of skin bearing the particular modified scales were removed and fixed in cold (4°C) modified Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde) (Humason, 1962). Material for wax embedding was subjected to routine histological procedure, using paraffin wax (Paraplast: Sherwood Medical Co.) as embedding medium. Sections were made on a Reichert-Jung rotary microtome at 10μ, and put on glass microscope slides. In addition, some specimens were embedded in plastic and sectioned at 6μm. All sections were made in series,

and stained with Harris' haematoxylin and counter stains (eosin, azan or PAS) (Humason, 1962) and viewed under a Leitz Laborlux 12 light microscope.

Serial sections were made both laterally and longitudinally, enabling visualization of gross morphology, histological description of all parts of the generation gland and identification of areas of activity. Skin scales adjacent to generation gland were staged, using the methods described by Maderson (1966), see Table 1. The same system was used for staging generation glands. The serial sections were made through entire glands, and in most cases the counter stains were rotated so that each consecutive slide was coloured with a different stain.

2.3. RESULTS

2.3.1. CORDYLID SKIN HISTOLOGY (PERFECT REST STAGE AND STAGE 4)

a) *Perfect Rest Stage (PRS)*: This is indicated by the presence of an incomplete skin generation, consisting of a syncitial β-layer/ oberhautchen complex, on top of an complete immature α-layer, all of which overlies the inactive SG. Histologically, the syncitial β-layer/ oberhautchen complex is an amorphous chromophobic layer, with no cellular boundaries or organelles visible. No mesos layer was observed in any section of over 150 specimens sectioned in all three species (Figure 2a). Rather the situation appears to be that α-layers cells get progressively more compressed as presumptive α-layer cells are produced below the existing α-layer cells. The presumptive α-layer cells are more basophilic than presumptive β-layer cells, and are more laterally flattened, with a distinctive elongated ovoid shape. They have large nuclei and a granular cytoplasm. The mature α-layer cells become flattened, losing organelles and nuclei. They are also chromophobic in H&E, although they do stain positive for PAS. The most mature α-layer cells eventually become nothing more than compressed membranes. The SG is inactive in PRS and the cells appear flattened.

b) *Stage 4*: For simplification of comparison between gland types, generation glands compared display renewal cycle stage four (Table 1). To this end, a histological description of stage four in normal skin scales follows. Stage four is described as having a complete outer generation (oberhautchen, β-layer, α-layer, lacunar tissue layer, clear layer) and an incomplete inner generation

consisting of an incomplete oberhautchen, β- and α-layers (Figure 2b). Thus the outer generation is the same as that found in PRS, with the addition of the lacunar tissue layer and clear layer below the now mature α-layer. The lacunar tissue layer is the only layer of all six to have visible nuclei in the mature state, as all other layers nuclei become pycnotic. Cells of lacunar tissue layer have a poorly basophilic and strongly eosinophilic cytoplasm, and are less flattened than α-layer cells. The clear layer cells are more rounded and smaller than the lacunar tissue layer cells, with a slightly stronger basophilic reaction, but can only really differentiated because the oberhautchen cells of the inner layer lie below them. The clear layer is only one cell layer thick, and immediately overlies the oberhautchen of the inner generation.

The oberhautchen of the inner layer is also a single row of cells. During stage four, the oberhautchen and the β-layer of the inner epidermal generation have not yet formed a syncitium. Individual oberhautchen and β-layer cells can still be seen. The cells of the maturing oberhautchen are large and oval, with large slightly flattened nuclei, and stain particularly well with PAS. The presumptive β-layer cells are extremely flattened, and lie at an oblique angle, orientating toward the posterior apex of the outer scale surface. Their nuclei are extremely flattened and pycnotic, and the cytoplasm is densely packed with large basophilic granules. The presumptive β-layer of the inner generation is much thicker that the β-layer of the outer generation in stage four. Below this β-layer, the α-layer is formed. Presumptive α-layer cells are distinctly different from the presumptive β-layer cells. They are also flattened, but much shorter and thicker, with very thick cell membranes. They stain in a similar manner to the presumptive β-layer cells with eosin, but with PAS, they stain much lighter.

The SG is actively producing new cells to create the new epidermal generation. The cells are cuboidal to columnar, with very large nuclei, almost filling three-quarters of the cell area. The cytoplasm does not stain very positively with PAS, but slightly better with eosin, and the nuclei are strongly basophilic.

2.3.2 GROSS MORPHOLOGY OF CORDYLID EPIDERMAL GLANDS

The typical condition of the femoral gland is that displayed in *C. cordylus* (Figure 2c). The entire structure of the femoral gland can be seen, showing the

femoral gland body, surrounded by the envelope which separates the gland proper from the dermis above it and the muscle it overlies (puboischiotibialis muscle). The femoral gland consists of the femoral gland body, or gland proper, as well as the pore canal and the opening o the femoral gland pore in the middle of the femoral gland pore scale. The scale immediately proximal to this on the ventral surface of the thigh is the generation gland. These generation glands are easily identified under low magnifications due to the enlarged β-layers in the build-up of generations. This also shows how normal epidermal scales surround these specialized epidermal derivatives.

2.3.3. DETAILED MORPHOLOGY OF CORDYLID EPIDERMAL GLANDS

Single-layer Stacked Generation Gland (*Pseudocordylus capensis*):

The specialized portion of the scale lies in the centre region of the outer scale surface. The rest of the scale consists of unspecialized skin layers. The enlargement of the β-layer means that even with only one mature generation, the glandular material protrudes above the level of surrounding normal skin scales (Figure 2d). Stereo-zoom examination using a dissection microscope, shows the glandular area is in a roughly circular area close to the posterior apex of the outer scale surface (OSS). The differentiated area appears milky white to grey, compared to the brown appearance of the unspecialized scales. The glandular area can vary in size, sometimes covering one third of the scale surface, and at other times encompassing virtually the entire scale surface, leaving the unspecialized skin visible only at the hinge region (Figure 2e).

Under light microscopy, the histology of the unspecialized portion of the generation gland (that region surrounding the specialized area) was seen to be the same as that of unspecialized epidermal scales of the same renewal stage. The stage of renewal of the generation gland is the same in both the glandular portion of the scale and the unspecialized epidermis on the peripheral region of the scale. Normal, unspecialized epidermal scales are all synchronous with each other, whereas the generation glands are at different stages of renewal from these surrounding normal scales (Figure 3 a & b). The occurrence of this asynchrony was noticed in all but six of 143 individual specimens of all three species sectioned.

There is a clearly demarcated boundary area between normal and differentiated β-material. It is visible as a horizontal "Y"-shaped structure composed of normal β-layer/oberhautchen syncitial material, with the open arms of the "Y" bracketing the periphery of the differentiated β-layer. The arm proximal to the SG is longer than the arm distal to the SG. Sometimes the distal arm is non-existent, i.e. the undifferentiated β-layer comes to an abrupt halt, while the proximal arm can underlie the differentiated material for some distance. This structure (Figure 3c) extends all around the periphery of the glandular area. Normal mature β-keratin is chromophobic, thus the bracket structure is virtually colourless, while the differentiated β-layer stains positive with eosin, azan or PAS. This further emphasizes the bracket area. The other skin layers remain continuous below this structure, as only the cells of the β-layer differentiate to produce the glandular exudates, and in doing so, visually change structure.

Generation glands' outer generations are not sloughed off at the end of each shedding cycle as is the case on the normal, unspecialized scales. The complete outer generation overlies the skin below over the entire surface of the generation gland. The unspecialized skin of the peripheral region of the generation gland remains connected to the specialized glandular portion of the generation gland, but is separated from the skin of the surrounding unspecialized scales (Figure 3d). Unspecialized skin of the generation gland appears to split from surrounding skin scales in the hinge region, indicated by the length of the piece of skin that overlies the new skin generation. The glandular material is in the centre of the scale and it is to only this portion of the skin that the unspecialized skin remains attached. The unspecialized skin border has the appearance of a mantle or blanket that surrounds the glandular portion of the scale. The change from synchronous and asynchronous renewal appears to occur in the hinge area. Histologically, the unspecialized 'mantle' is identical to the complete unshed normal outer epidermal generation of unspecialized epidermal scales (Figure 4a). It possesses all the layers present in normal skin (see 2.3.1.), including the oberhautchen. The mid-portion of the generation gland in contrast (Figure 4b) displays only differentiated epidermis with a glandular β-layer and no oberhautchen. The dermis below the gland is well vascularized. The SG is columnar and has a basophilic cytoplasm and nucleus.

The presumptive α-layer (Figure 4c) lies above this, and consists of at least four layers of dorso-ventrally flattened, oval cells, with large nuclei and few granules in the cytoplasm. These cells have basophilic nuclei and cytoplasmic inclusions that are eosinophilic. There is no visible mesos layer, merely a progression of gradually more flattened α-cells until the presumptive β-layer (pβi) begins. This boundary is marked by a cessation of dorso-ventrally flattened cells, and the beginning of laterally flattened cells. These are the differentiated presumptive β-cells. They are so tightly packed, and extremely laterally flattened, that there appear to be many layers of these cells. Closer investigation shows that there are only two or three layer of cells. The nuclei lie at different levels, furthering the illusion of multiple layers. The cells are not vertical, instead orientate at a slight angle toward the posterior apex of the outer scale surface (OSS). The nuclei in this layer that lie proximal to the SG are rounder and show mitotic activity. The nuclei that lie distally to the SG are flattened and show virtually no mitotic activity, and appear pycnotic. This layer is less basophilic and has more cytoplasmic inclusions than in the α-layer. Differentiated glandular β-cells stain more positively with counterstains (eosin, azan and PAS) than does the α-layer. PAS stains this presumptive β-layer particularly well.

The mature outer epidermal generation overlies the presumptive β-layer (pβi), and consists of a layer of reduced living cells, the clear layer, followed by the lacunar tissue layer, where most of the cellular structure is gone, leaving only fibres, which are clearly visible when the skin is stretched too far by histological procedures (Figure 4d). The mature α-layer is visible as a dark band, with only a few highly reduced, flattened nuclei. All cells lie extremely close together, and no cell boundaries can be seen, only a pattern of horizontal bands, that are either fibres or thickened cell membranes of extremely flattened cells. The cell membrane is all that remains, staining well with PAS and azan, although eosin doesn't stain this layer well unless the sections were over-stained. All these layers are similar those found in normal skin. The β-layer differs from normal skin, because the differentiated cells have an amorphous appearance. The nuclei are all broken down, as are the cell membranes. The cytoplasm has a highly granular appearance, and retains the shape of the old cell, even though the membrane is gone. Stretching and splitting of the layer by either histological procedure or shedding, results in cracks in this layer,

where the cells retain their shape while being pulled apart from each other. The mature β-layer layer stains well with counterstains, most vividly with azan. Sometimes there are portions of the β-layer that retain the staining intensity with PAS similar to that of the presumptive β-layer. These portions are always in the middle of the gland and in the area proximal to the α-layer (Figure 4e).

Multiple-layer Stacked Gland (*Cordylus cordylus*):

Similar to the single-layer stacked gland, the differentiated portion of the scale is situated in the middle of the scale, adjacent to posterior apex of the OSS, and is surrounded by unspecialized skin. The glandular area appears white to yellow on external observation and covers at least half of the OSS (Figure 5a). Some scales, particularly those in the middle of the gland patch, have almost the entire scale surface taken up by differentiated tissue, leaving only a small peripheral area of unspecialized skin adjacent to the hinge region.

The obvious difference between the single- and multi-layered stacked glands is the number of mature generations stacked above the presumptive generation in the multi-layered stacked gland. Male *C. cordylus* have stacks varying from 5 to 12 generations in height. These protrude up to six times as high above the SG than surrounding unspecialised scales (Figure 5b). Another noticeable difference is the thickness of the presumptive β-layer (pβi) of the glandular portion. This does not appear to result in similar thickness' of β-layers in resulting mature generations as all specimens sectioned showing stage 4 had thick pβi's, but uniformly thick mature β-layers of approximately two-thirds the thickness of the pβi (Figure 5c).

The unspecialized skin on the periphery of the multi-layered stacked gland is also stacked up in multiple generations just like the specialized glandular portion. The peripheral skin is not anchored in the hinge area, and hangs loose, attached only to the glandular portion of the mature generation. The mature generations are moved further from the SG by new generations forming and pushing the old ones up. The mantle is also pushed up by the same procedure, and since it is thinner than the glandular portion of the generation gland, as the mantle layers are observed to stay on the side of the glandular stack. The unspecialized skin keeps to the contours of the stack, curling out at the ends, forming a multi-layered 'mantle' over the sides of the stack of mature generations (Figure 6a). Some of the complete generations of unspecialized skin can be absent on the outermost generations of

the stacked gland, due to abrasion (Figure 6b).

Histology and staining intensity of this scale-type is identical to the single-layer stacked gland. The periphery of the glandular area is still marked by the "Y-bracket". The unspecialized peripheral skin of the generation gland is at the same stage of renewal as the differentiated glandular region. This is generally at a different stage to that displayed on surrounding unspecialized scales. The mid-portion of the glandular area and the mature generations (Figure 6c) are histologically the same as the single-layer stacked gland, there are simply more generations in the multi-layer generation gland. The unspecialized skin of the periphery of the generation gland has the same histology as that found in *P. capensis*. The mature generations of the unspecialized skin, stacked above each other, are all the same length. This indicates that the zone between synchronous and asynchronous renewal lies in the hinge area between scales (Figure 5b).

Multiple-layer Pit Gland (*Pseudocordylus microlepidotus*)

Stereo-zoom investigation shows a similar picture to that of all cordylid generation glands investigated. The scale has glandular material in the centre of the OSS. This material is different in colour (milky-white to yellow), texture (flaky or sandy), and sheen (dull and non-reflective) to the surrounding unspecialized scales (Figure 7a).

Gross morphology is very different from the previously described generation glands. The differentiated portion is found in the middle of the scale, as in the other types of generation glands, but here the similarity ends. The SG is deeply invaginated, lying below the level of that of surrounding skin scales. The number of pits varies between glandular scales, with some scales having only one pit, and others up to three or four pits. The histology and morphology within each pit is identical. The build-up of generations rarely protrudes above the level of the perimeter of the pit (Figure 7b). Production of glandular material takes place on the bottom of this pit. The entire SG of the scale is active (Figure 7c), and producing cells of skin generations. SG on the sides and top of the pit results in unspecialized skin, which builds up in thick layers on the side of the pit. Most of the resulting mature generations on the top of the scale, peripheral to the pit, appear to have been abraded away. The top layer of the glandular material generations also has

an abraded appearance, with cells appearing to terminate in straight lines (Figure 7d). The morphology of the unspecialized skin on the top and periphery of the scale is the same as that described for the unspecialized peripheral skin in the previous two scale types (cf. Figure 8 with Figures 3b & 7c). The morphology of the glandular area within the pit is unlike the generation glands previously described.

The interior of the pit is not a perfect round hole. There are folds of dermis that protrude into the pit from the sides and bottom of the pit. These dermal folds vary in size and shape between specimens and could possibly vary between adjacent generation glands. The fold protrudes into the pit from the side and base, and may extend completely across the pit in the plane of the femur. Dermal folds may extend all the way to the surface of the scale in some specimens, anchored at the sides and base of the pit. Some specimens may only have folds that reach halfway across the pit or to the surface; in other specimens, these dermal folds are not present at all. There are sometimes large blood vessels in these folds, but most of the dermis in these folds is occupied by normal loose connective tissue (Figure 7b) typical of the dermal area adjacent to the SG.

New glandular material is formed at the base of the pit. If dermal folds are present, the glandular material is formed in bottom of the resulting troughs. Adjoining troughs may have varying stages of shedding in the same glandular scale, although this difference is slight and is probably due to the centrifugal renewal gradient found over the OSS of any scale (Maderson pers. comm.). In such a situation, staging should reflect the most advanced area of epidermis, i.e. that found in mid-sagittal axis of the scale. Scales with multiple pits appear to show a mini centrifugal gradient in each pit. There is the overall gradient for the scale as a whole, but within each pit there appears to be areas that are slightly more advanced than others and these areas are all located slightly towards the posterior apex of the scale, just off the middle of each pit

The histology and staining intensity of this scale-type is identical to both of the previous gland types. Generation glands in this species are also asynchronous from the surrounding unspecialized epidermal scales.

2.4. DISCUSSION

This study has shown that cordylids have similar skin histology to that found in gekkonids (Maderson, 1967; 1968a & b; 1972). All the skin layers comprising a complete generation of unspecialized skin are present and visible in cordylids except the mesos layer.

Further similarity between cordylids and gekkonids exists in the resemblance of the generation glands of cordylids to the beta-glands of gekkonids, in that both cordylid generation glands and gekkonid beta-glands produce glandular material via a differentiated β-layer (Maderson, 1970). Although there are three types of gland in cordylids, (all three producing glandular material in the β-layer (van Wyk & Mouton, 1992)) the greatest similarity exists between the cordylid single-layer stacked gland (*P. capensis*), and the beta-glands of gekkonids (Maderson, 1968b). In both types, there is only one mature layer of glandular material present, the oberhautchen is not continuous over glandular area, and the glandular material is of β-layer origin.

The classification system for Cordylidae generation glands (van Wyk, 1997a) highlighted two main types of generation glands, namely pit glands and stacked glands. Before this, cordylid generation gland types were divided in to three, using the same basic types, but subdividing the stacked gland into single- and multi-layered stacked glands (Van Wyk & Mouton, 1992). This appears to be the best classification system available, particularly in light of the conformity of these gland types within species.

P. capensis is a much more active lizard than *P. microlepidotus* or *C. cordylus* (Branch, 1988). In *P. capensis*, it is possible that either communication is more visual than chemical, or that chemical material is produced more rapidly (i.e. shedding is more frequent) than the other two species. As this species is so active, friction from the substrate could abrade away mature generations, thus physical pressure is another possibility for only one mature generation remaining on *P. capensis* generation glands

The only gross morphological difference between *C. cordylus* and *P. capensis* is that *C. cordylus* shows vertical stacking of the multiple generations over the generation gland. Other than this, there is no morphological or histological

variation between these species. The reason for the stacking in *C. cordylus* is as unclear as the lack of stacking in *P. capensis*. It is possible that *C. cordylus* has a higher turnover or production rate of skin generations over the generation gland than *P. capensis*. Because there is no information about how long it takes for a single shedding cycle over a generation gland in any cordylid, it is not possible to determine turnover rates, and any conclusions drawn about this would be speculation.

P. microlepidotus shows, as in *C. cordylus*, variation from *P. capensis* in that there are multiple generations stacked on top of each other over the generation gland, although these multiple generations are invaginated below the outer scale surface, while those of *C. cordylus* protrude above the outer scale surface. With all cordylid generation gland types investigated, there are no additional or unique layers when compared to that unspecialized skin. The cells are similar to those present in unspecialized skin, except for the β-layer, which is differentiated to produce the glandular material. Previous investigation (van Wyk & Mouton, 1992) postulated that this gland type was related to the escutcheon scale of gekkonids, due to the production of glandular material in a concavity, but the production of glandular material via a β-layer makes the pit gland much closer to the beta-glands of gekkonids. It is difficult to say whether these pit glands are simply inverted stacked glands, although there is indication that growth progresses downwards during the generation glands existence. The pit appears to be continuously increasing in either depth or shape. Mature glandular generations appear to retain the shape they were produced in, giving a representation of what the SG used to look like when the generation was formed. Multiple generations do not always have the same contours, especially if they are split by dermal folds. Thus, solid mature generations overlying split mature generations would indicate the dermal fold came into existence after the solid mature generation was created. This indicates that the glands grows deeper with time, as the oldest mature generations may be whole mature generations, whereas younger mature generations may be split by dermal folds. Growth cannot continue above the SG, as the complete layers cannot be formed from an SG already split by a dermal fold. What would appear to be the case is that the dermal folds grow up with generations surrounding them, until they

reach the surface, where abrasion and friction will stop them growing any further. Multiple pits on a single generation gland thus appear to have been formed by dermal folds that have reached the surface, and stretch across the entire glandular scale.

P. microlepidotus showed a large amount of variation in gross morphology (viz. varying heights of dermal folds, multiple pits/single pits), although this did not result in different histology or cytology. The use of serial sections showed these variations not to be a result of artefacts created by histological procedure. This variation could be due to a result of a lack of standardization concerning the choice of generation glands used for investigation in *P. microlepidotus*. Only adult male lizards were used for this study. These were all caught from coastal montane areas, with similar weather conditions. Further research needs to be done on variation in generation glands on single individuals. As adult lizards age, they develop more generation glands (Mouton & van Wyk, 1993). This does not result instantly in a mature, multiple-layered gland; rather these layers are built up over time.

It is likely that the original form of the gland was the simplest: the single-layer stacked gland, which became a multiple-layered gland by the evolution of stacking. Neither of the remaining cordylid generation gland types can be said to be more advanced than the other, as parallel development from a common ancestor could result in both the pit gland and the multi-layer stacked gland. The pit gland possesses more adaptations (viz. dermal folds, multiple pits and stacking) when compared to the multi-layer stacked gland (viz. merely stacking), but any declaration that one is more advanced would be mere conjecture.

The multiple pits that pit-glands display provide interesting information, particularly with regard to the evolution of femoral glands. Several possible paths of evolution from unspecialized epidermal scales to functioning femoral glands have been postulated. The most parsimonious solution is that femoral glands are derived from generation glands that have become asynchronous with surrounding skin scales and invaginate below the dermis (Maderson, 1970; Maderson & Chiu, 1970). This study has shown that cordylid generation glands operate out of synchrony with surrounding skin scales, thus, part of the hypothesis is completed. Pit glands display invagination, although this is not below the level of the dermis like the gland proper of the femoral gland. Growth was also shown to continue in a downward

manner, i.e. the gland is constantly invaginating, although no specimens were shown to invaginate below the level of the dermis. If one were to postulate that the multiple pits created by the dermal folds could develop continuous production of glandular material instead of the production of modified skin generations, then each pit would be analogous to a follicle in the femoral gland proper. The dermal folds portioning the scale into multiple pits would then be analogous to the follicular membranes surrounding the follicles in the femoral gland.

The variation between the single-layered stacked gland (*P. capensis*) and multiple-layered pit gland (*P. microlepidotus*) appears to corroborate with the suggestion that *Pseudocordylus* is not a monophyletic group (Frost et al., 2001). Their study showed that *P. capensis* and *P. nebulosus* are separated from the group containing *P. microlepidotus*.

There is little generation gland information to date. This is due to so few species having been histologically investigated for the presence of glands. The gekkonids and cordylids are the only families thus far that have been positively identified as having generation glands (Maderson, 1970; van Wyk & Mouton, 1992), and these have now both been investigated using detailed histology. This study has highlighted the need for detailed histological investigation because until recently, external examination of generation glands had assumed that while generation glands were present on cordylids, they were all the same type.

Table 1: The six renewal stages of the generalized skin shedding cycle in squamates (Maderson, 1985; Maderson et al., 1998). Table showing the different skin cell types in squamate epidermal generations and the presence or lack thereof at each stage of renewal (Abbreviations).

Renewal Stage	SG State	Inner Generation				Outer Generation					
		αi	mesos	β_I	Obi	clo	lto	αo	mesos	βo	Obo
Stage 1	Inactive	not present	not present	not present	not present	not present	not present	present, mature	present, mature	present, together form syncitium	
Stage 2	Active	not present	not present	not present	present, presumptive	present, presumptive	present, presumptive	present, mature	present, mature	present, together form syncitium	
Stage 3	Active	not present	not present	present, presumptive	present, presumptive	present, presumptive	present, presumptive	present, mature	present, mature	present, together form syncitium	
Stage 4	Active	present, presumptive	present, presumptive	present, presumptive	present, presumptive	present, presumptive	present, presumptive	present, mature	present, mature	present, together form syncitium	
Stage 5	Active	present, presumptive	present, mature	present, together form syncitium		present, mature	present, mature	present, mature	present, mature	present, together form syncitium	
Stage 6	Active	present, mature	present, mature	present, together form syncitium		present, mature	present, mature	present, mature	present, mature	present, together form syncitium	

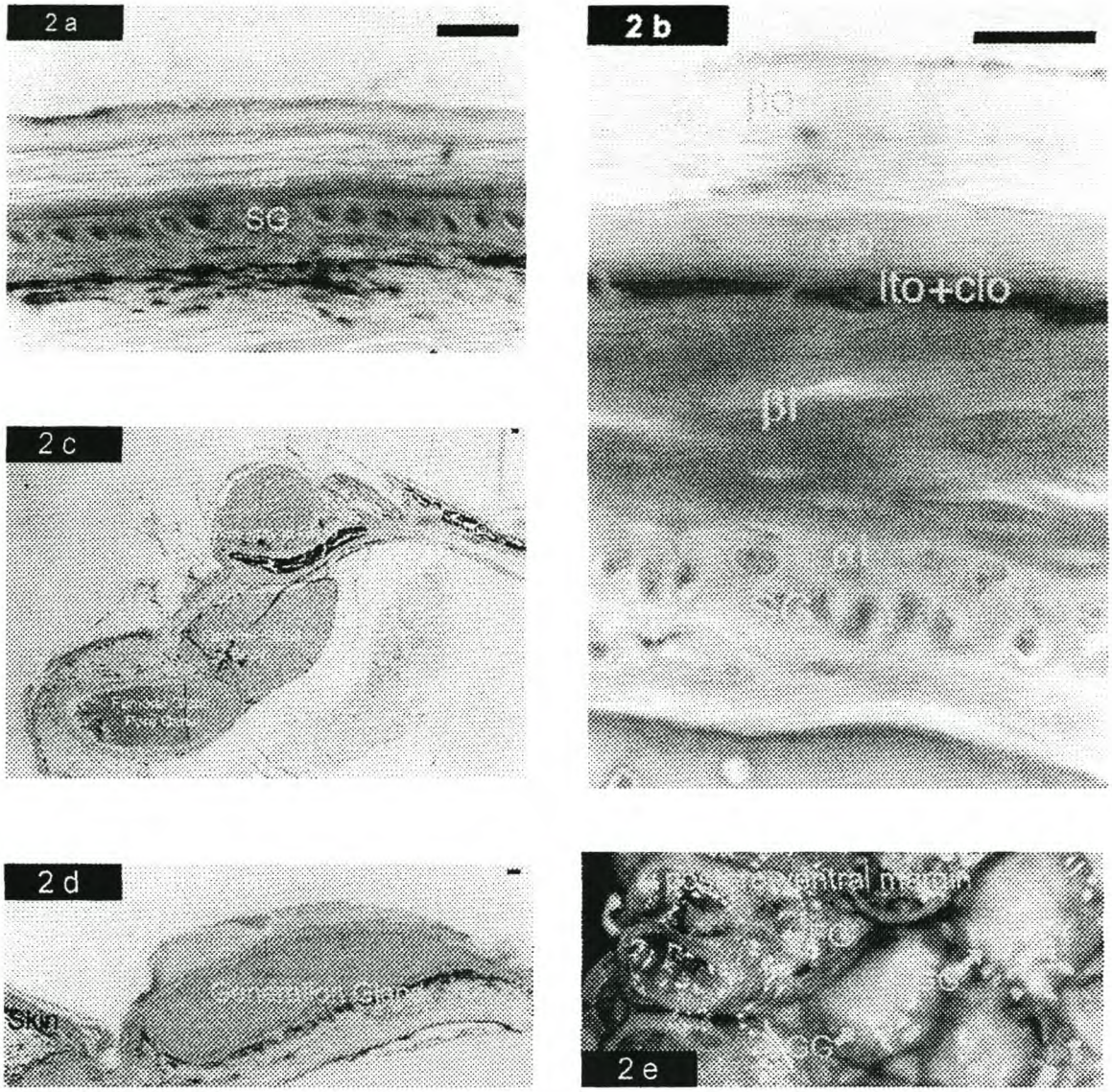


Figure 2, Skin scale and generation gland micrographs of cordylid lizards: a) Unspecialised skin scale displaying PRS; b) Unspecialised skin scale displaying skin renewal stage four; c) Thigh region of *C. cordylus*, showing the position of the femoral gland relative to generation glands and unspecialized skin; d) *P. capensis* single-layered generation gland, the gland protruding above surrounding skin scales; e) Stereozoom micrograph of thigh of *P. capensis* displaying the positions of the various skin scale derivatives: Femoral glands (FG), generation glands (GG) and the postero-ventral margin of the thigh . scale bar = 1000μm

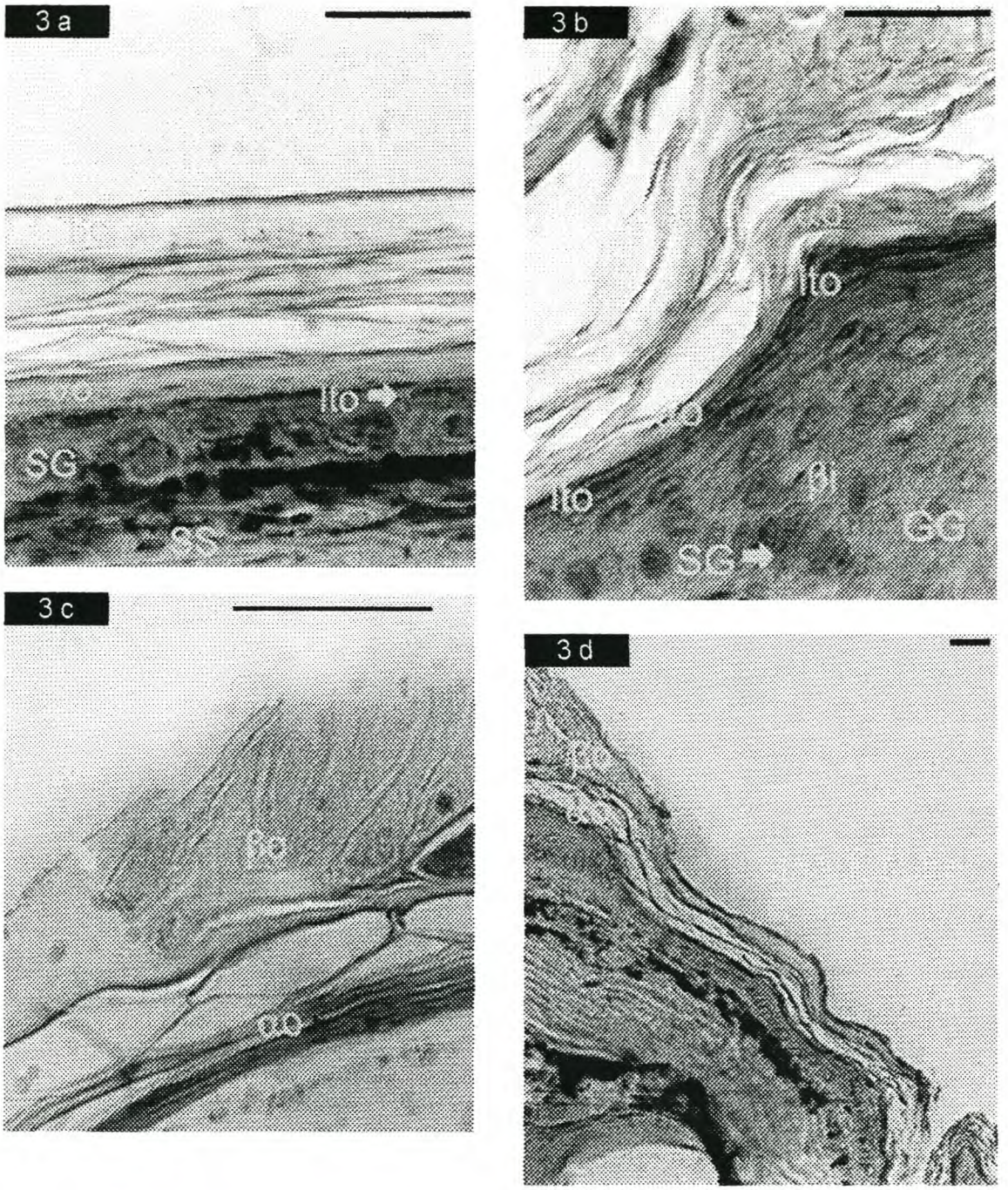


Figure 3, Skin scale and generation gland micrographs of cordylid lizards: a) Unspecialized skin scale displaying PRS; b) Margin of generation gland from same individual as 3a, with both unspecialized skin of periphery of generation gland, and glandular material producing area displaying skin renewal stage 3; c) Unspecialized skin bordering the differentiated skin of the glandular area, displaying the "Y-bracket" of β-material; d) Peripheral skin of the generation glands remains unattached at the hinge region. scale bar = 1000 μm

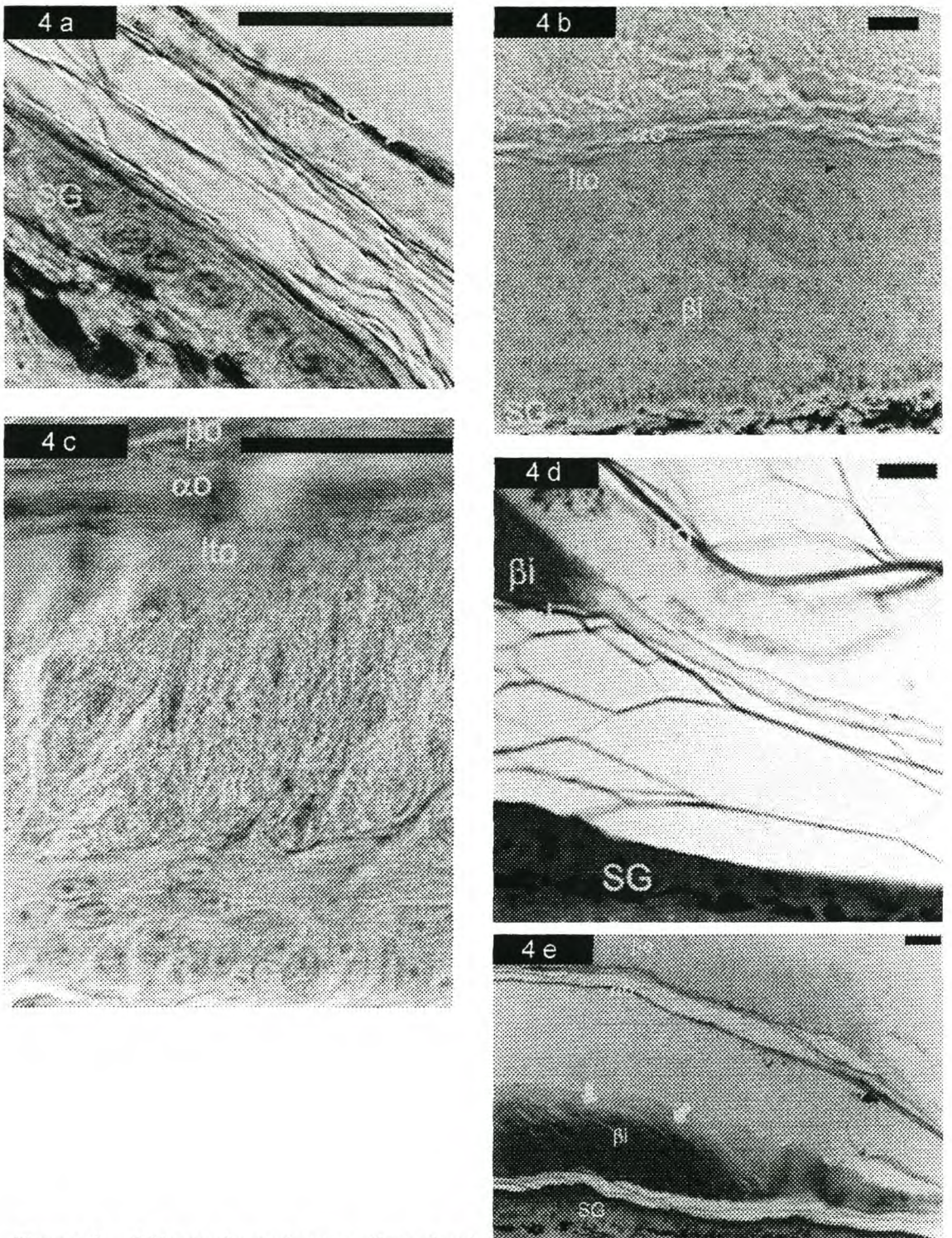


Figure 4. Skin scale and generation gland micrographs of cordylid lizards: a) Skin peripheral to the glandular material on the generation gland; b) Mid-portion of the glandular scale, displaying differentiated skin; c) Germinative layer and newly produced skin layers, of the glandular region of the generation gland (skin renewal stage 4); d) Generation gland that has been pulled apart by histological procedures, displaying the fibres of the mesos-layer and lacunar tissue; e) *P. capensis* generation gland, displaying variation in PAS staining intensity. Arrows illustrate where the stain is concentrated. scale bar = 1000 μm.

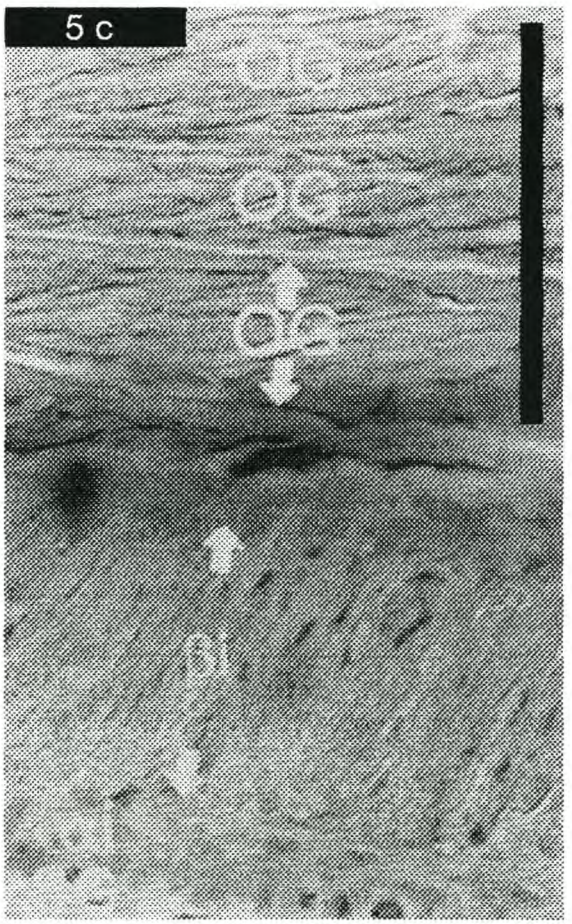
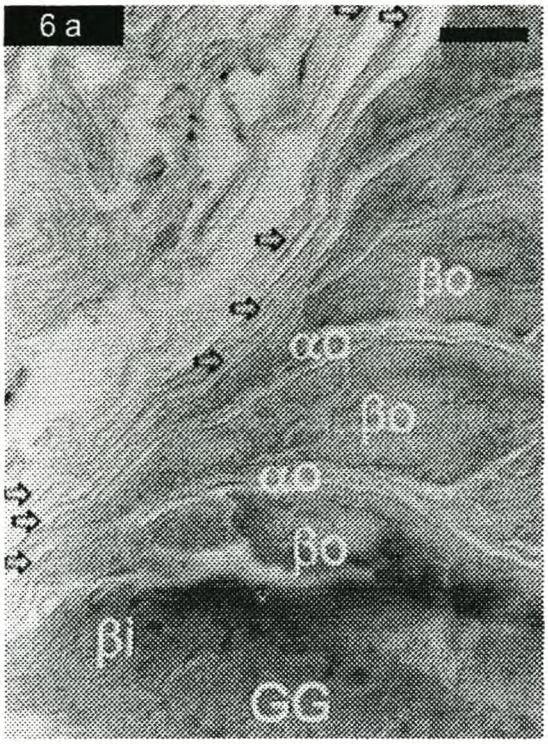
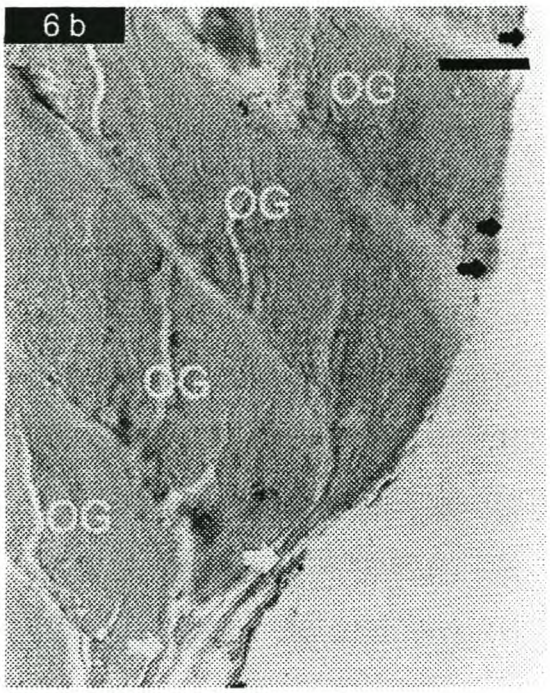


Figure 5, Skin scale and generation gland micrographs of cordylid lizards: a) Stereozoom micrograph of the thigh of *C. cordylus*, displaying the positions of the skin scale derivatives relative to each other; b) *C. cordylus* generation gland, displaying the height of the stack of Outer Generations (OG) above the surrounding skin scales; c) *C. cordylus* generation gland, showing how the presumptive β-layer is twice as thick (indicated by arrows) as the resulting mature β-layers. scale bar = 1000 μm.





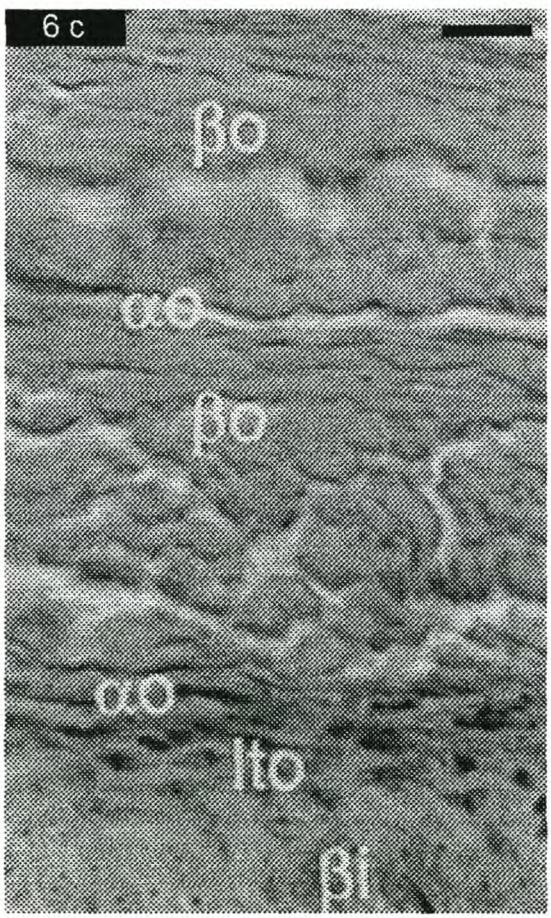


Figure 6, Skin scale and generation gland micrographs of cordylid lizards: a) Multi-layered 'mantle' (indicated by the black arrows) over the side of mature glandular generations; b) Top and side of typical C. cordylus generation gland, displaying marks of abrasion; c) Mid-portion of the glandular area and the mature generations are histologically the same as the single-layer stacked gland. scale bar = 1000 μm.

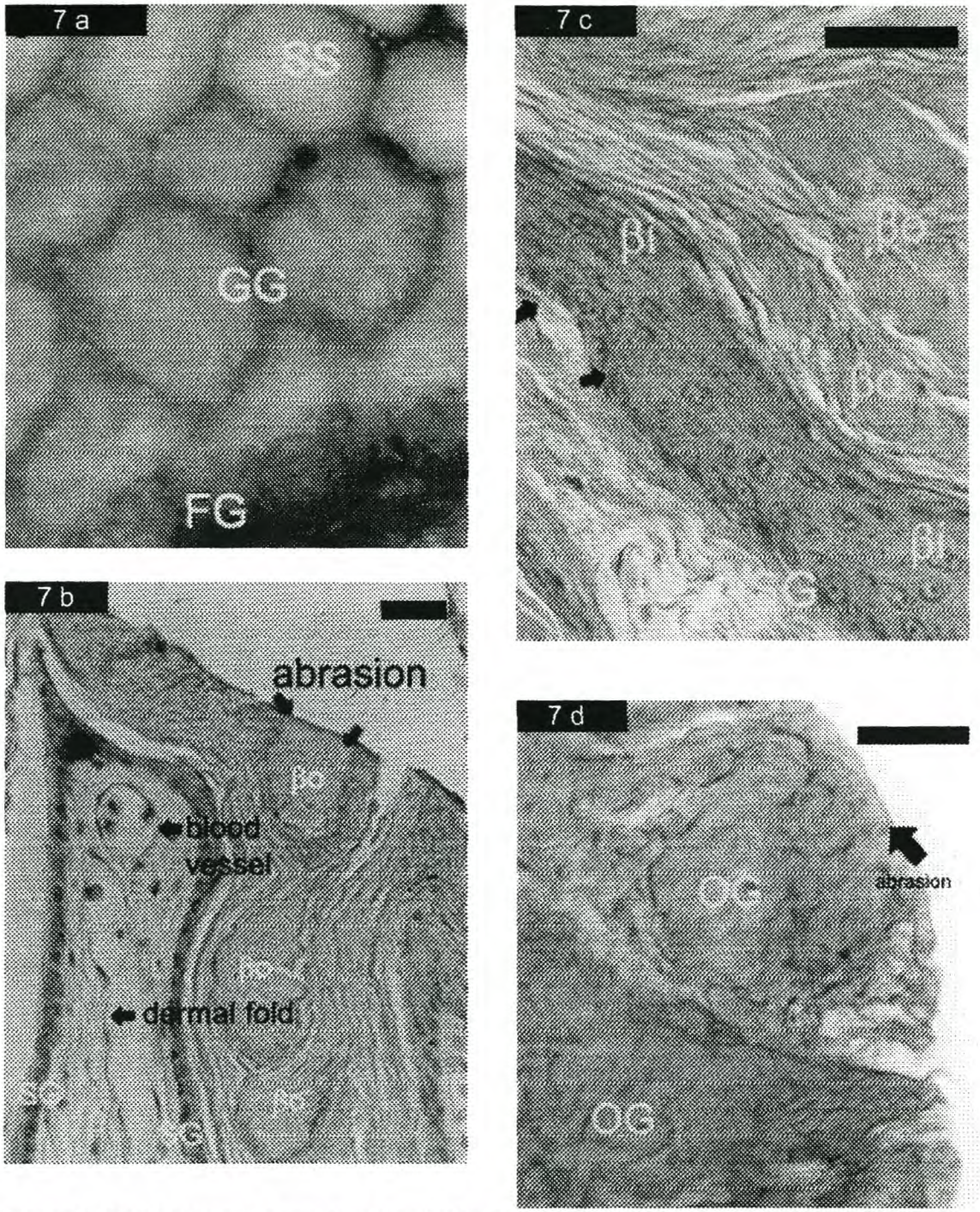


Figure 7. Skin scale and generation gland micrographs of cordylid lizards: a) Stereozoom micrograph of underside of thigh of *P. microlepidotus*, showing position of all skin scale derivatives relative to each other; b) Cross-section through *P. microlepidotus* pit-type gland. Glandular material does not protrude above the level of the pit due to abrasion; c) Side and bottom of a *P. microlepidotus* pit-type gland. SG actively proliferating, only the base of the pit makes glandular material. The area between the arrows is where this changeover occurs; d) Glandular material of generation gland displaying an abraded appearance, with cells appearing to terminate in straight lines. scale bar = 1000 μm.

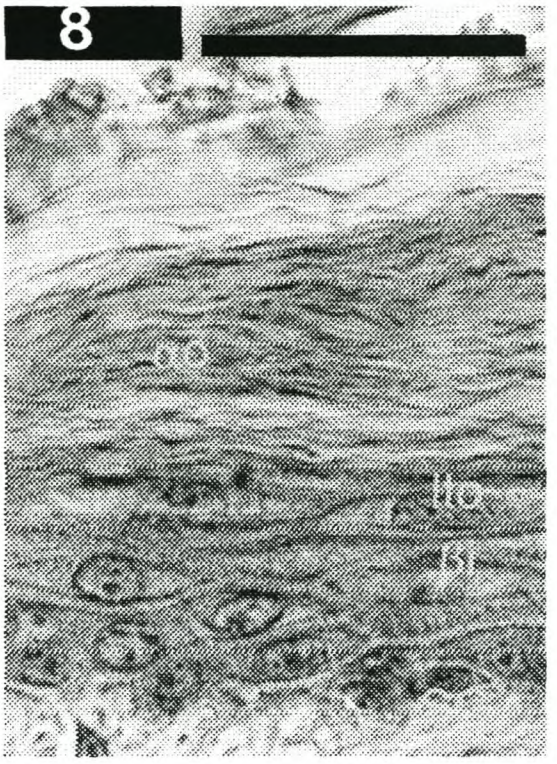


Figure 8. Peripheral unspecialized skin on the generation gland scale, showing renewal stage three. scale bar = 1000 μm.

THREE

SHEDDING CYCLE HISTOLOGY AND SEASONAL ACTIVITY OF SKIN AND GENERATION GLANDS IN THREE SPECIES OF CORDYLID LIZARDS (SAURIA: CORDYLIDAE) DISPLAYING DIFFERENT TYPES OF GENERATION GLANDS

3.1 INTRODUCTION

The outer surface of the amniote epidermis consists of dead cells or cellular derivatives produced by the living cells of the deepest epidermal layer, the stratum germinativum (Spearman, 1973). As these epidermal cells age, they differentiate and die, leaving on the body surface a protective layer composed mostly of the fibrous protein keratin (Irish et al., 1988). All amniotes renew epidermal tissue throughout life. In amniotes other than reptiles, this proliferation can be described as individual mature epidermal cells produced asynchronously from the body surface, that are lost either as single cells or single units (large flakes, feathers or hairs) (Maderson et al., 1998). Cells in such an epidermis are produced by the SG, move upward due to more cells being produced below them, pass through a series of cytodifferentiative steps, mature and then are ultimately lost to the external environment. Only a single type of mature keratinocyte can be defined (Maderson et al., 1998).

Skin proliferation in squamates, in contrast, is a cyclical process synchronized over the entire body surface. It has long been documented that snakes shed their skin *in toto* or in large pieces, in fact, ever since the Greek myths. When a lizard or snake loses its skin, it loses an entire epidermal generation. This generation is a complex of six different types of keratinocyte, each with different cytological characteristics (Maderson et al., 1998). Lizard epidermis has been histologically described in several studies (Maderson, 1965; 1966; 1967; Maderson et al., 1972; 1998). The clearest description of a "generalized" reptilian epidermis is provided by Maderson (1966; 1985) and Maderson et al. (1998). The lost generation consists of six layers that vary in thickness. These layers are, from the outermost layer to the SG: oberhautchen, β-layer, mesos layer, α-layer, lacunar tissue- and clear layers.

During shedding, a complete mature epidermal generation is shed from the body and a generation comprising only an α-layer, mesos layer and β-layer is left behind. This new incomplete generation characterizes the rest phase. This rest phase has been sub-divided into a 'post-shedding rest stage', a 'perfect rest stage' and a 'late rest stage'. The duration of these three stages varies interspecifically, and can be days, weeks or even months (Maderson, 1985). No cellular proliferation or cellular differentiation is visible in this stage, which ends when the proliferative activity of the renewal phase resumes. The remaining layers of the outer generation are formed during the renewal phase, until the outer generation is complete. The renewal phase continues to produce the oberhautchen, β-layer and a partial α-layer of the inner epidermal generation (Maderson et al., 1972). When shedding occurs the clear layer of the outer epidermal generation separates from the oberhautchen of the inner epidermal generation. This process as a whole is referred to as the shedding cycle. Renewal phase lasts approximately 14 days in all species studied thus far (Maderson, 1985).

Generation glands are intra-epidermal glandular specializations found on postero-ventral aspects of the thigh or abdomen of two families of lizards (Gekkonidae and Cordylidae) (van Wyk & Mouton, 1992). Gekkonids have been widely investigated and both of their types of generation glands have been histologically described (Maderson, 1967; 1968a). In both cases, the glandular material is exposed to the environment after shedding. Due to the high shedding rate, as short as 25-35 days for a complete skin shedding cycle in *Gekko gecko* at constant temperatures and photoperiod (Chiu & Maderson, 1980), this can result in frequent renewal of glandular material.

Epidermal glands are derivatives of the reptilian skin, thus should in theory be affected to some degree by the hormones that usually regulate the skin's behaviour (Maderson, pers. comm.). These glands are also used as secondary sexual characteristics in many taxonomic descriptions (FitzSimons, 1943; Loveridge, 1944; Lang, 1991), implying that the effects of sex hormones should influence them. The majority of research on generation glands relates to the influence of sex hormones on the epidermal glands (Chiu et al., 1970; Maderson & Chiu, 1981).

Information on cordylid skin morphology and shedding is scanty. All evidence about cordylid shedding cycles is unconfirmed, but observation of captive cordylids and some unpublished data has indicated that cordylid shedding is likely to be an annual cycle (van Wyk; pers. comm.; Visagie, pers. comm.). Previous research on cordylid skin has been limited to skin derivatives, namely femoral and generation glands (van Wyk & Mouton, 1992; van Wyk, 1990, 1997a & b; van Wyk et al., 1997).

In chapter two, data is presented about the three known types of generation glands in Cordylidae. These are pit glands, single-layer stacked glands and multi-layer stacked glands. It is still unclear how the number of generations on these gland-types varies so greatly, and it could be hypothesized that each gland-type is associated with a different shedding cycle, or shedding cycle frequency. The objectives of this study are twofold. Firstly, to describe the cytological changes during the various stages of the cordylid shedding cycle for both unspecialized skin and generation glands in representative species of Cordylidae, each with one of the different gland types. Secondly, to try to ascertain any temporal or seasonal aspects of the shedding cycle of unspecialized skin or generation glands in these species.

3.2 MATERIALS AND METHODS

To describe the shedding cycle, skin samples were taken from the ventral region of the right thigh, in an area that would encompass femoral glands as well as generation glands, and unspecialized skin scales. Femoral glands were removed in order to orientate the skin sample after histological procedure. Species representing the various generation gland types were used in this experiment, namely *Pseudocordylus capensis* (single-layer stacked gland), *Cordylus cordylus* (multi-layer stacked gland) and *P. microlepidotus* (multi-layer pit gland).

Samples were taken from specimens from J. Ellerman Museum of the Zoology Department at the University of Stellenbosch. These samples were removed and placed in 70% ethyl alcohol. Because the museum material was collected in different months, this provided an opportunity to get preliminary seasonal data.

Some additional specimens were collected in April 1998 from each species by noosing, sacrificed by lethal injection of sodium pentabarbitone and skin samples were taken from the right thigh. The skin sample was taken from the area including the glandular patch, in order to section generation glands together with normal skin scales in one series. The skin samples were removed and fixed in cold (4 °C) modified Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde). All material was subjected to routine histological procedure, using paraffin wax (Paraplast: Sherwood Medical Co.) as embedding medium (Humason, 1962). Sections were made on a Reichert-Jung rotary microtome at 10μ, and placed on glass microscope slides. All sections were made in series, starting at one side of a generation gland and sectioning until the other, and stained with either Harris' haematoxylin and eosin, azan or PAS (Humason, 1962) and viewed under a Leitz Laborlux 12 light microscope. Serial sections were made in order to provide histological description of all parts of the generation glands and normal unspecialized scales, and to allow identification of areas of activity. Skin scales adjacent to generation glands were staged using the methods described by Maderson (1966), and the same system was used for staging modified scales (Table 1).

3.3 RESULTS

3.3.1 DESCRIPTION OF THE CYTOLOGICAL STAGES DURING THE SHEDDING CYCLE OF UNSPECIALIZED SKIN IN CORDYLIDAE.

The descriptions that follow are based on *C. cordylus*, with references to any differences in the other two species, *P. capensis* and *P. microlepidotus*.

Stage one (Figure 9a, b & c)

Immediately after shedding, the cordylid epidermis is composed of two distinct parts, an outer generation (OG) and a basal SG. The outer generation is incomplete, i.e. it does not consist of all six layers present in generalized epidermal generations. The incomplete OG characterizing this stage in cordylids consists of the oberhautchen, β-layer, α-layer and one layer of living cells, the SG. Very few spines on the oberhautchen are visible at 1000x magnification. The β-layer is a chromophobic, homogenous, keratinized layer of cells, overlaid by the spinulate oberhautchen. Together, these form a syncitium, which is sometimes separated from the α-layer

below it. This separation is not a shedding break, rather an artefact of histological procedure. In this region of histological splitting, fine fibres can be seen. Theoretically, this region would be the mesos layer in unspecialized skin. Below this, the α-layer is visible. Mature α-layer cells stain pink with eosin, or violet with PAS, and this part of the α-layer has a similar appearance to the β-layer, namely that the cells are flattened, have thick cell walls and a lamellate appearance. Below the mature α-layer cells is one row of presumptive α-layer cells. These are not as flattened as mature α-layer cells and stain very in the same way to the SG. They contain large nuclei and a finely granular cytoplasm. SG cells are at in rest phase, according to the staging (Table 1), and the cells are few in number, flattened or cuboidal, with small, basophilic nuclei. Immediately before the proliferative stages (2-6) begin, the layers of the OG are produced, although not all are mature. The oberhautchen/β-layer syncitium is already mature. All presumptive α-layer cells become keratinized, flatten and lose nuclei, completing the mature α-layer. The lacunar tissue layer is produced below the α-layer. Presumptive lacunar tissue cells are polygonal and basophilic. The clear layer is formed below this, and is composed of one row of cells that are elongated and stain lightly with all stains, although show a preference for haematoxylin. Thus, all the layers of a complete generation are present. Mitotic activity can be seen in some SG nuclei.

Stage two (Figures 10a, b & c)

This is the first stage of the proliferative period of the skin shedding cycle. The epidermis is now composed of an immature, complete OG, presumptive cells of the inner generation (IG), and an actively proliferative SG.

The OG is composed of (1): mature oberhautchen/β-layer syncitium, fine fibres that could be the mesos layer and the mature α-layer; and (2): immature lacunar tissue- and clear layers. All staining and morphological aspects of the OG are the same as those encountered in the pre-shedding phase of stage one, except that the lacunar tissue cells become more flattened. The incomplete IG is represented by presumptive oberhautchen cells. These cells appear as laterally flattened shapes, with large nuclei, which are also laterally flattened. The cells have an affinity for eosin and PAS, but not such a good affinity for azan. SG cells are

cylindrical, numerous and tightly packed, with large basophilic nuclei that occupy over 50% of the cell volume and weakly eosinophilic, granular cytoplasm.

Stage three (Figures 11a, b & c)

Gross morphology reveals a complete, immature OG, an incomplete, immature IG, and an active SG. The OG shows no changes from that in stage two, except that the clear layer is now differentiated into a thin keratinized layer. The IG is now composed of a presumptive oberhautchen and a presumptive β-layer. The production of the β-layer characterizes stage three. Four to seven rows of living, presumptive β-layer cells are seen under the oberhautchen, which are horizontally flattened, with many pycnotic nuclei. The cytoplasm of these presumptive β-layer cells contains many granules, and stains darker with PAS than the presumptive oberhautchen cells.

Stage four (Figures 12a, b &c)

Stage four is identified by formation of the presumptive α-layer of the inner generation. The epidermis consists of an immature, complete OG, an immature, incomplete IG and an active SG. The OG shows no change from that in stage three. The IG consists of the oberhautchen and β-layer, and the SG produces a new layer. Keratinization progresses down toward the SG through the β-layer (IG). Cell walls increase in thickness as they keratinize, and their pycnotic nuclei begin to disappear. β-layer cells closer to the SG have thinner cell walls and pycnotic nuclei. As stage four progresses, all β-layer cells become keratinized and lose nuclei
The IG develops a new layer; the cells of which are much smaller than those in the presumptive β-layer of the IG. The cytoplasm is granular, but the granules are finer than those in the β-layer cell's cytoplasm. This new layer consists of about five layers of cells, and these stain particularly well with PAS but not as well with eosin or azan. Maderson et al. (1972) indicated that presumptive mesos cells could be differentiated from α-layer cells by the fact that mesos cells do not keratinize, and in 1985, he indicated that the presumptive mesos layer is only 3μm thick in *Gekko gecko*. As all of the cells below the β-layer appear to keratinize, this new layer must be the α-layer.

Stage five (Figure 13a, b &c)

The OG is the same as described in stages three and four, immature and complete. The inner generation does not develop a new layer in this stage; rather the existing layers differentiate further. This stage is characterized by the formation of the oberhautchen/β-layer syncitium (IG). Both of these layers are now fully keratinized, and now lose all visible cells boundaries and form a thick composite layer that is identical in all respects the oberhautchen/β-layer syncitium (OG). This new syncitium loses all affinity for any stain and becomes chromophobic. The presumptive α-layer continues to be produced. Fine fibres are visible between the β-layer and α-layer of the (IG) when these two layers are separated by histological procedures. α-cells closest to the β-layer become keratinized and lose nuclei, and as keratinization progressed downward toward the SG, more α-cells begin to develop pycnotic nuclei. The α-layer continues to be produced even though the peripheral parts of the layer (those furthest from the SG) are fully mature.

Stage six (Figure 14a, b & c)

The shedding complex (clear layer of OG and oberhautchen of IG) splits, and separates the now mature, complete OG from the incomplete, immature IG. The SG is slightly reduced, and the cells vary from being cylindrical to cuboidal in shape. All OG layers are now mature. The lacunar tissue layer is now thicker than in previous stages, as it becomes swollen, and it stains darkly with PAS and haematoxylin. The clear layer of the OG is, as in stages four and five, a thin keratinized layer, although as it is split from the oberhautchen of the IG, it sometimes fragments along vertical lines. It stains well with azan, but is not clearly differentiated from the lacunar tissue layer when stained with PAS or eosin.

The inner generation is the same as seen in stage five, as is consists of a mature oberhautchen/β-layer syncitium and an incomplete α-layer. Presumptive α-layer cells are still produced, and older α-layer cells become keratinized and form part of the incomplete mature area of the α-layer. The α-layer stains positive with PAS, but appears chromophobic when stained with azan or eosin.

The only difference visible between species is the presence of osteoderms in the dermis of *C. cordylus*. These bony plates are flattened, and lie in the deep

fibrous dermis below the SG. *P. capensis* and *P. microlepidotus* have no osteoderms.

3.3.2 DESCRIPTION OF THE CYTOLOGICAL STRUCTURES DURING THE STAGES OF THE SHEDDING CYCLE OF GENERATION GLANDS IN CORDYLIDAE.

General Observations

In order to compare generation glands with skin scales, generation glands are described according to the six stages of renewal previously used (Table 1). Generation glands do not show the same cytology over the whole scale surface, as is the case in unspecialized skin scales. Glandular material is only produced by differentiated skin in the centre of the outer scale surface (OSS) in stacked glands, and in the bottom of the pit, which is also in the centre of the OSS in pit glands. This differentiated area is surrounded by unspecialized skin (Figure 15c, d, e, f). Both glandular and unspecialized epidermis on the generation gland display the same stage of skin renewal (Figure 15c & d), but the generation gland displays a different stage of renewal from surrounding unspecialized skin scales (Figure 15e & f). The peripheral unspecialized areas of the generation gland types differ from typical unspecialized skin scales in that mature generations do not shed off, but remain attached to the glandular portion of the generation gland. They are unattached at the hinge area, and form a type of mantle around the glandular portion, which stacks up on top of the epidermis. This results in a multi-layered portion, which stacks up on top of the epidermis. In multi-layered generation gland types, this results in several generations of unspecialized skin stacked on top of each other (Figure 16a).

The descriptions that follow will describe the cytological structures of each shedding stage (Table 1). Only one OG will be described, as the cytology does not vary between mature OG's. Once an OG is mature, it does not differentiate any further (Figure 16a).

Stage one (Figure 16b)

The epidermis consists of a complete OG (or OG stack in the case of multi-layered gland types) and an active SG. Non-proliferative SG cytology was never

encountered in any section through any generation gland. The cells are cylindrical, tightly packed, with large basophilic nuclei and a non-granular cytoplasm. The glandular OG consists of (from distal to proximal to SG): a differentiated β-layer, α-layer, lacunar tissue layer and clear layer. The β-layer is the layer that is differentiated to produce glandular material. In contrast to the lamellate, chromophobic oberhautchen/β-layer syncitium encountered in unspecialized skin, glandular, or unspecialized β-layers have: (1) no oberhautchen on the surface of the β-layer; (2) no syncitial structure; (3) positive staining characteristics for all counterstains used and (4) a different cellular orientation, namely obliquely orientated toward the posterior apex of the OSS. These mature glandular β-layer cells do not keratinize; they have pycnotic nuclei, and a granular cytoplasm that stains positive for all counterstains used. The cellular orientation is difficult to see in mature cells as cell membranes have broken down to release the glandular material of the cytoplasm. Some areas of the β-layer are slightly pulled apart along oblique lines, which appear to be cell boundaries these separations indicate cellular orientation. Adjacent unspecialized β-layers of the peripheral unspecialized skin of the generation gland have the same cytology as that observed in typical unspecialized skin's β-layers of the same stage (cf. Figure 9a).

The α-layer is visible as a thin lamellate band underlying the β-layer. There are no intercellular structures visible (no nuclei or cytoplasmic organelles), merely a flattened mass of thick keratinized cell membranes. The α-layer stains positive with PAS, and appears chromophobic when stained with eosin or azan. Presumptive α-layer cells are laid down below the mature α-layer, in a similar manner to that seen in unspecialized skin both on peripheral unspecialized skin of the generation gland and in unspecialized skin scales of the same renewal stage (cf. Figure 9a).

Peripheral unspecialized skin, surrounding the glandular area of the generation gland has identical histology to that observed in typical unspecialized skin of the same stage.

Stage two (Figure 17a & b)

The epidermis consists of an immature, complete OG, with the lacunar tissue layer and clear layer being produced in the terminal phases of stage one. These are both in a presumptive state in stage two, with the lacunar tissue layer being

polygonal and basophilic, while the clear layer is formed below this, composed of one row of cells that are elongated and stain lightly with all stains, although show a preference for haematoxylin. The glandular β-layer of the OG does not vary from the situation described in generation glands' stage one.

The active SG produces the first layer of modified β-layer cells of the IG in this stage. The cells are oval, laterally flattened, with large basophilic nuclei and granular cytoplasmic inclusions that stain positive for PAS, eosin and azan.

Unspecialized skin on the periphery of the glandular area of the generation gland shows the same cytology as that observed in unspecialized skin scales displaying the same skin stage.

Stage three (Figure 17c, d & e)

The glandular OG remains unchanged from the situation observed and described in stage two for generation glands, except that the clear layer is differentiated into a thin keratinized band. The active SG produces several more rows of presumptive β-layer cells: oval, laterally flattened, tapering at both ends, with large basophilic nuclei and granular cytoplasmic inclusions that stain positive for PAS, eosin and azan. The presumptive β-layer cells furthest from the SG begin to differentiate: nuclei become pycnotic and more granules develop in the cytoplasm, thus the cytoplasm stains much darker with PAS, azan and eosin. The differentiation starts at the cells most distant from the SG, progressing downward toward the SG.

Unspecialized skin on the periphery of the glandular area of the generation gland shows the same cytology as that observed in unspecialized skin scales displaying the same skin stage.

The two different β-layer types can clearly be seen in the boundary area between glandular and unspecialized skin, where the differentiated glandular β-layer meets the unspecialized β-layer of the peripheral skin. This is often visible as a Y-shaped structure in *C. cordylus*, although this structure is much clearer as both of these layers are differentiated fully, and the unspecialized oberhautchen/β-layer syncitium forms a Y-shaped structure (Figure 3c).

Stage four (Figure 18a, b & c)

The OG is now mature except for the lacunar tissue layer which is still presumptive and made up of polygonal cells. Similar to unspecialized skin, this stage is characterized by presumptive α-layer cells being produced by the active SG. These presumptive α-layer cells are the same as those occurring in normal skin. The cytoplasm contains many fine granules and consists of about five layers of cells, which stain particularly well with PAS but not as well with eosin or azan. The α-layer of the glandular area of the generation gland appears the same as that in the peripheral unspecialized skin, and no differences can be seen. The β-layer continues to differentiate, with many more granules appearing in the cytoplasm. The cytoplasm of the glandular β-layer cells of the IG stains very well with PAS, especially when compared to that of the mature β-layer cells of the OG.

Unspecialized skin on the periphery of the glandular area of the generation gland shows the same cytology as that observed in unspecialized skin scales displaying the same skin stage.

Stage five (Figure 19a, b & c)

The OG remains the same as that observed in stage four. The β-cells of the inner generation differentiate further and lose cell membranes in this stage. The shape of the cell is retained, as can be seen in areas that have split during histological preparation. Nuclei become pycnotic or disappear completely. Differentiation continues as can be seen by different intensities of stain reaction to PAS. Presumptive α-layer cells are produced below the existing α-layer, with the typical finely granular cytoplasm of presumptive α-layer cells.

Unspecialized skin on the periphery of the glandular area of the generation gland shows the same cytology as that observed in unspecialized skin scales displaying the same skin stage.

Stage six (Figure 20a & b)

The OG is now complete, and fully mature. The lacunar tissue layer is now much thicker, due to swelling of the cells. Shedding would occur along the zip-fastener mechanism between clear layer (OG) and oberhautchen (IG) except there is no oberhautchen. Some localized separation is visible between the inner and outer generations, although the separation is not complete over the whole gland.

Presumptive α-layer cells are still produced. The β-layer is now completely differentiated, which no cell membranes, no nuclei, and a cytoplasm that consists of many large granules. This layer as a whole has an amorphous appearance.
Throughout all stages, marks of abrasion can be seen on the surface of the β-layer of the outermost OG. The tapering ends of the β-cells are absent, apparently abraded along a straight plane that covers the entire surface of the generation gland (Figure 7d).

3.3.3 SEASONAL FREQUENCIES OF SHEDDING ACTIVITY IN BOTH UNSPECIALIZED SKIN SCALES AND DIFFERENTIATED GLANDULAR SCALES (GENERATION GLANDS)

P. CAPENSIS (SINGLE-LAYERED STACKED GLAND)

Data regarding shedding activity in unspecialized skin appears to indicate one period of active renewal per annum (Figure 21a), thus showing a seasonal pattern of skin shedding activity. Early skin renewal stages (1-3) are seen between November and December, and between February and April. No specimens displayed advanced skin renewal stages (4-6) in this period. Advanced skin renewal stage 6 was seen in both January and August. No data is available for the period between April and August due to the inaccessibility of specimens.

Generation gland histology showed no discernable pattern of seasonal activity on a histological level (Figure 21b). No extended periods of inactivity (i.e. PRS) like that shown in the unspecialized skin were detected. Only one specimen displayed stage one, although this was late stage one, an active renewal stage. All stages of skin renewal, both early and advanced, were arranged haphazardly all through the year. Months in which many specimens were sectioned had nearly all the skin renewal stages represented.

C. CORDYLUS (MULTI-LAYERED STACKED GLAND)

Unspecialized skin also showed a distinct seasonal pattern of skin shedding activity, with a long period of epidermal inactivity (no renewal stages) between March and August (Figure 22a). This coincides with the autumn and winter seasons. Renewal stages (2-6) were observed from September to February, indicating one period of epidermal renewal. Early renewal stages (1-3) were observed between September and December, and advanced renewal stages (5 & 6)

were observed in January and February. Early skin renewal stages (1-3) were observed throughout the renewal period, indicating that renewal does not occur simultaneously in all lizards of this species.

As in *P. capensis*, generation gland histology showed no discernable pattern of seasonal activity on a histological level (Figure 22b). No extended periods of inactivity (i.e. PRS) like that shown in the unspecialized skin were detected. All stages of skin renewal, both early and advanced, were arranged haphazardly all through the year. Months in which many specimens were sectioned had nearly all the skin renewal stages represented.

***P. MICROLEPIDOTUS* (MULTI-LAYERED PIT GLAND)**

This species' unspecialized skin's shedding cycle also displayed a seasonal pattern of skin shedding activity (Figure 23a), with an extended period of epidermal non-proliferation (PRS) starting in autumn (March), but this period is shorter than that observed in *C. cordylus* and ends in late winter (July). Thereafter the active renewal phases are evident, showing early skin renewal stages (1-3) from August until October, and advanced stages (4-6) from November until February. Stage six (actual shedding) was observed in two months, namely November (n=2) and February (n=1).

Generation glands show no seasonal pattern of skin shedding activity (Figure 23b). No specimens displayed PRS histology. Only one specimen had stage one skin renewal histology, and this was pre-shed histology, not PRS. No extended periods of inactivity (i.e. PRS) like that shown in the unspecialized skin were detected. All stages of skin renewal, both early and advanced, were arranged haphazardly all through the year. Months in which many specimens were sectioned had nearly all the skin renewal stages represented.

3.4 DISCUSSION

The different species appear to show different seasonal activity patterns with regard to the skin shedding cycles, in that the length of the non-proliferative period, when no renewal activity is noticed, varies considerably between species. The species with the longest non-proliferative period (six months), i.e. showing PRS, is *C. cordylus*, and *P. capensis* has the shortest period (two months) of epidermal inactivity. *P. microlepidotus* has a five-month period where no renewal activity is

noticed in the unspecialized skin. In all species, the period of epidermal inactivity is over the cooler months of the South African winter, while the skin displays epidermal renewal during the warmer months.

It has been shown in *Gekko gecko* that control of shedding may have to do in part with ambient temperature (Chiu & Maderson, 1980). In *Gekko gecko*, the warmer temperatures caused a reduction in the length of the rest phase or non-proliferative period between skin renewal, and a shortening of the time between skin-shedding events.

Since cordylid skin appears to have an extended period of rest, then starts renewal after several months, a stimulus of daylight length would presumably cause all animals in a population to initiate skin renewal at the same time. This is obviously not the case in *P. microlepidotus*, because 11 specimens were collected from one locality in one day in November, and of these: three displayed PRS histology, one displayed stage 2, one displayed stage 3, three displayed stage 4, one displayed stage 5 and two displayed stage 6. Daylight length appears not to be a stimulating factor for promoting the initiation of renewal, because if it were the stimulus, the renewal stages would all be synchronized throughout the population.

It is important to refer to renewal periods, as the data does not show how many cycles occur in the periods when renewal takes place. If the anecdotal evidence is correct, and cordylids do shed only once per annum (Mouton pers. comm.; van Wyk, pers. comm.), lizards living in colder climes may need longer to complete the proliferation necessary to produce the complete OG and incomplete IG. Data is not available about how long cordylids take to complete one shedding cycle, while it is known that gekkonids complete a renewal phase of a shedding cycle in 14 days (Maderson, 1985).

Generation glands were seen to be active all year round, displaying an asynchrony from the unspecialized scales. No rest phase was indicated in any species, i.e. no specimens displayed PRS histology in the generation glands. Stage one histology was observed in two specimens out of 146 sectioned, and this was late stage one (pre-shed phase), a renewal stage, not a rest stage. No patterns of seasonal activity were determined in generation glands of any species, illustrated particularly well by the fact that generation glands were actively undergoing renewal while the unspecialized skin was in a rest phase during cooler seasons. This was

indicated particularly well in *C. cordylus*, where 23 specimens showing PRS histology of the unspecialized skin in the autumn and winter months, and all these specimens' generation glands displayed one of the renewal phases (2-5) in an apparently random manner. The random arrangement of renewal stages in generation glands of all three species, combined with the seemingly seasonal arrangement of renewal stages, indicates strongly that generation glands operate out of synchrony with the surrounding skin scales.

This variation in seasonal activity between the scale types indicates that there is a pattern of continuous renewal on the generation gland. For this to be the case, a different method of controlling the differentiation of cell-types from the SG of the generation gland must be in place, as the unspecialized scales' SG lies quiescent while the generation gland is actively undergoing renewal.

Asynchrony between the specialized generation gland and unspecialized surrounding skin scales is easily observable, with a change in synchrony being easily identified in the hinge region between scales. The renewal stage of the layers above the SG is histologically distinctive, clearly showing skin renewal stages in adjacent scales changing in the hinge region. The length of the mantle layers can be compared to the length of the unspecialized skin peripheral to the glandular region, indicating that the unspecialized skin of the generation gland scale splits from unspecialized skin of adjacent unspecialized scales in the hinge region. Asynchrony and increased activity of generation glands can account for the rareness of times stage one was encountered in the generation glands of the three species. Assuming the generation glands are constantly active all year round, and that stage one is a rest phase, samples would rarely be taken in this short period in generation glands. This is what was encountered with only two out of 146 specimens showing stage one skin renewal stage in generation glands. This situation contrasts with that in the unspecialized skin scales where stage one is often encountered.

Van Wyk & Mouton (1992) speculated that in the case of *P. capensis*, with a simple generation gland, the situation may be similar to the Tokay gecko's beta-gland (Maderson, 1967), and that as stacking evolved in the cordylid generation gland, the frequency of gland shedding cycles increased. With no PRS seen on

generation glands, it certainly appears that these glands are very active, and frequency of shedding cycles over generation glands may have increased.

Van Wyk & Mouton (1992) indicated that asynchrony might exist between specialized glandular scales and normal, unspecialized skin. This has been shown, making Cordylidae the only family studied thus far to have asynchronous glandular scales.

Cordylidae are, in general, territorial animals (Branch, 1988). Chemical signals have been indicated to be included in femoral gland secretions of iguanids (Alberts, 1992). Such chemical signals are assumed components of generation gland secretions in cordylids, although experimental procedure when attempting to detect whether *C. cordylus* responds to chemical signals produced by con-specifics was not specifically targeted at specific gland types (Cooper et al., 1996). Specimens in this experiment reacted to scents that were deposited on substrate. These scents/signals could have been produced from a variety of sources: femoral glands, generation glands, cloacal glands, fecal boli or skin-derived semiochemicals. Further studies are needed to target specific chemical production sites.

In conclusion, Cordylidae display a seasonal pattern of shedding activity of unspecialized skin, with an extended period of skin non-proliferation (PRS histology) during cool periods. There is variation of this seasonal pattern in the different species, with *C. cordylus* having a longer non-proliferative period than either *P. microlepidotus* or *P. capensis*. The specialized, glandular skin scales, or generation glands, have a different shedding cycle activity pattern to that displayed by normal unspecialized skin, and thus display asynchrony with regard to skin shedding. These generation glands display no discernable pattern of seasonal activity on a histological level, and appear to be active throughout the year.

Asynchrony between generation gland and unspecialized skin poses some interesting questions for further research: how are these glands controlled, what is the significance of an asynchronous gland when compared to a synchronous gland, and why does Cordylidae possess such gland-types?

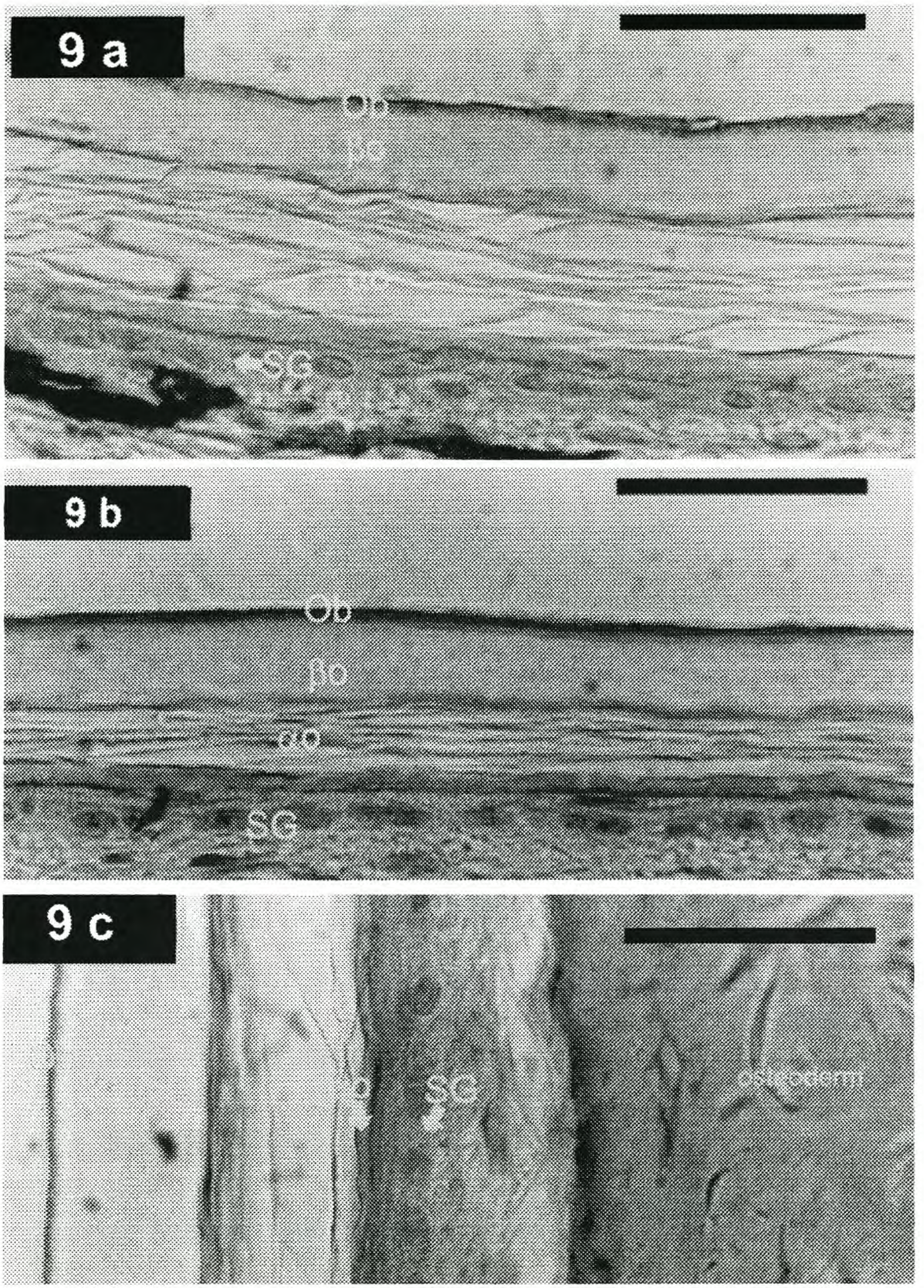


Figure 9, a) Unspecialized skin scales displaying PRS: a) of *P. microlepidotus*; b) of *C. cordylus*; and c) of *P. capensis*. scale bar = 1000 μm

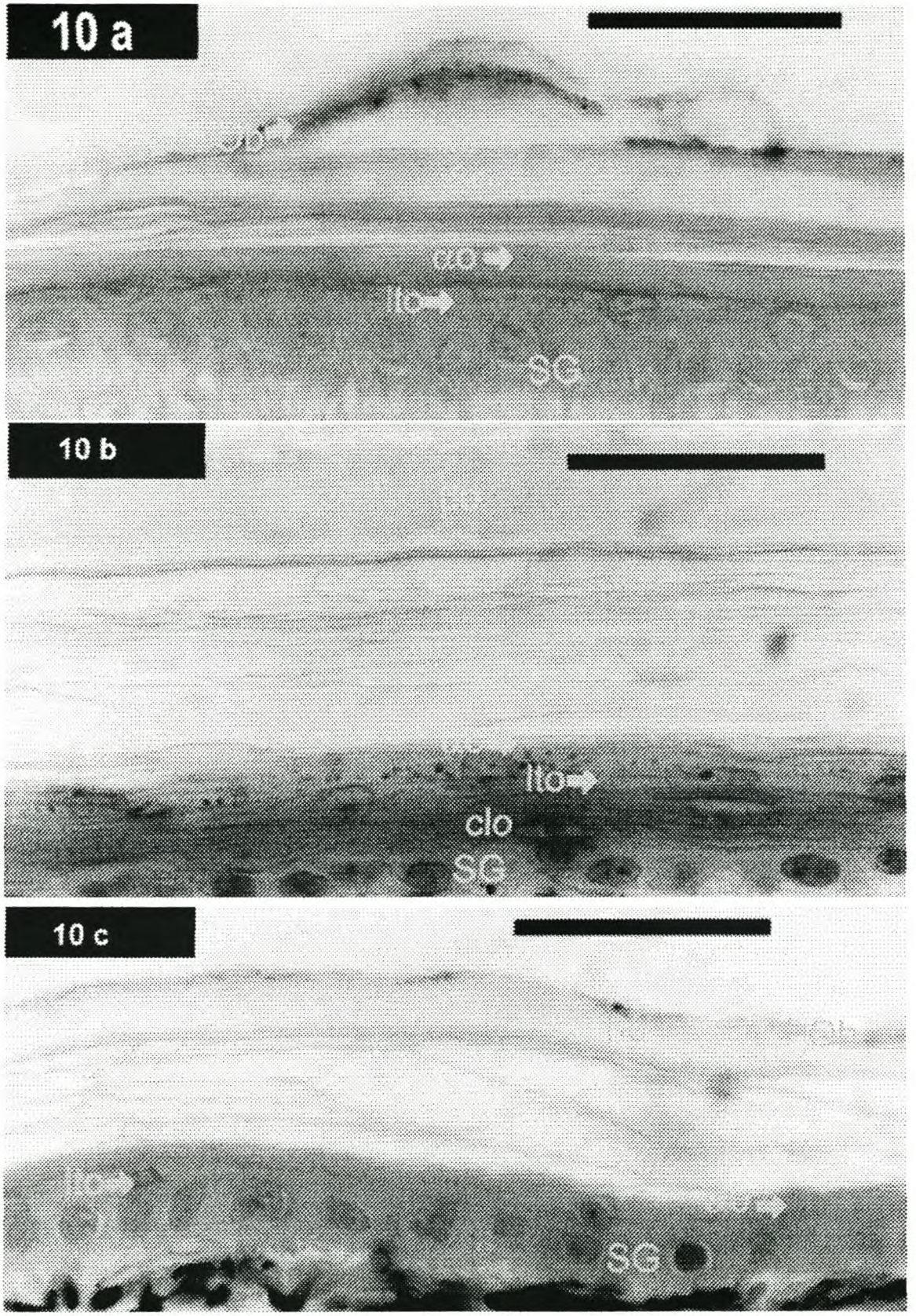


Figure 10. Unspecialized skin scales, displaying skin-renewal stage two of: a) *P. microlepidotus*; b) *C. cordylus* and c) *P. capensis*. scale bar = 1000 μm.

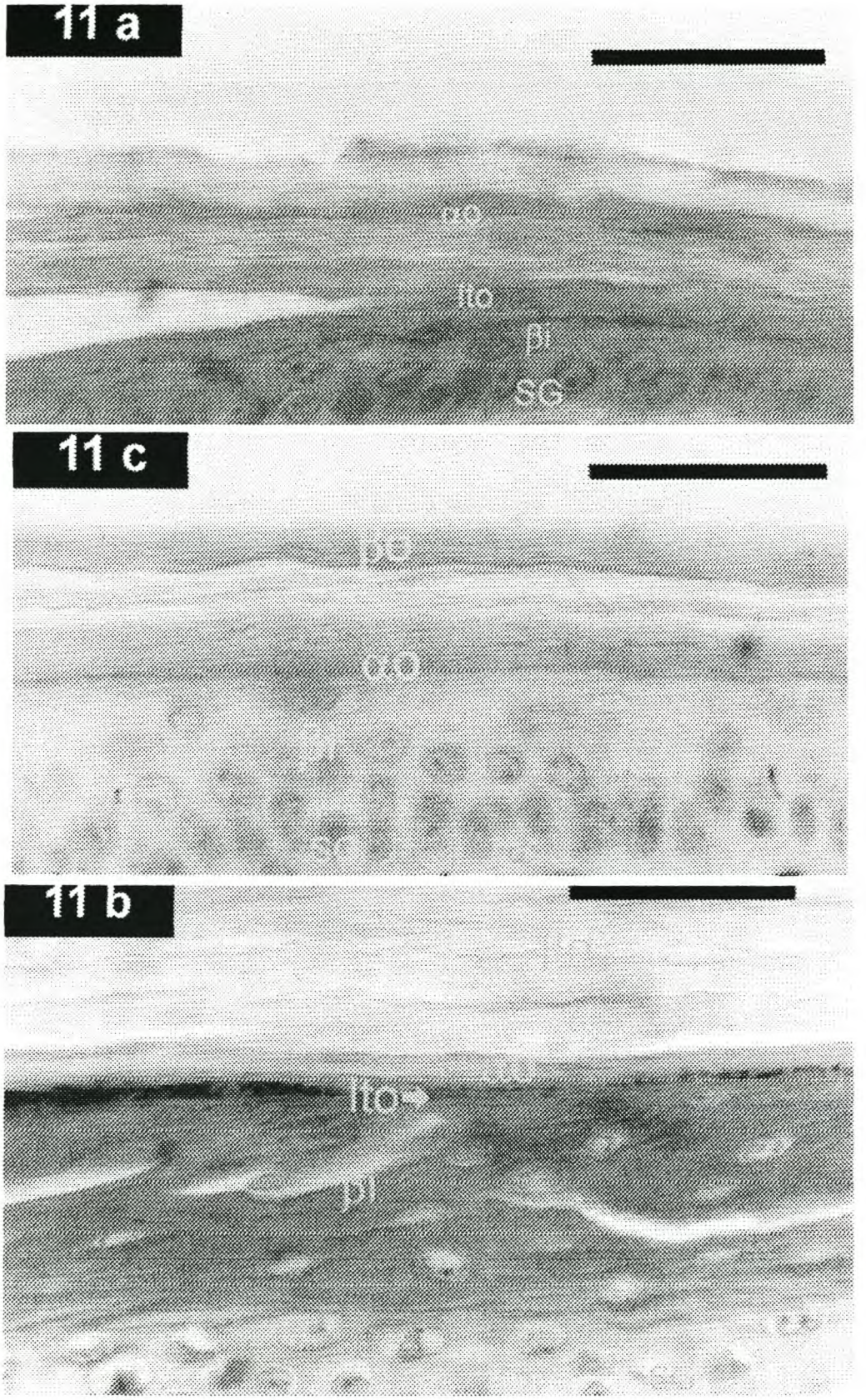


Figure 11 Unspecialized skin scales, displaying skin-renewal stage three of: a) *P. microlepidotus*; b) *C .cordylus* and c) *P. capensis*. , scale bar = 1000 μm.

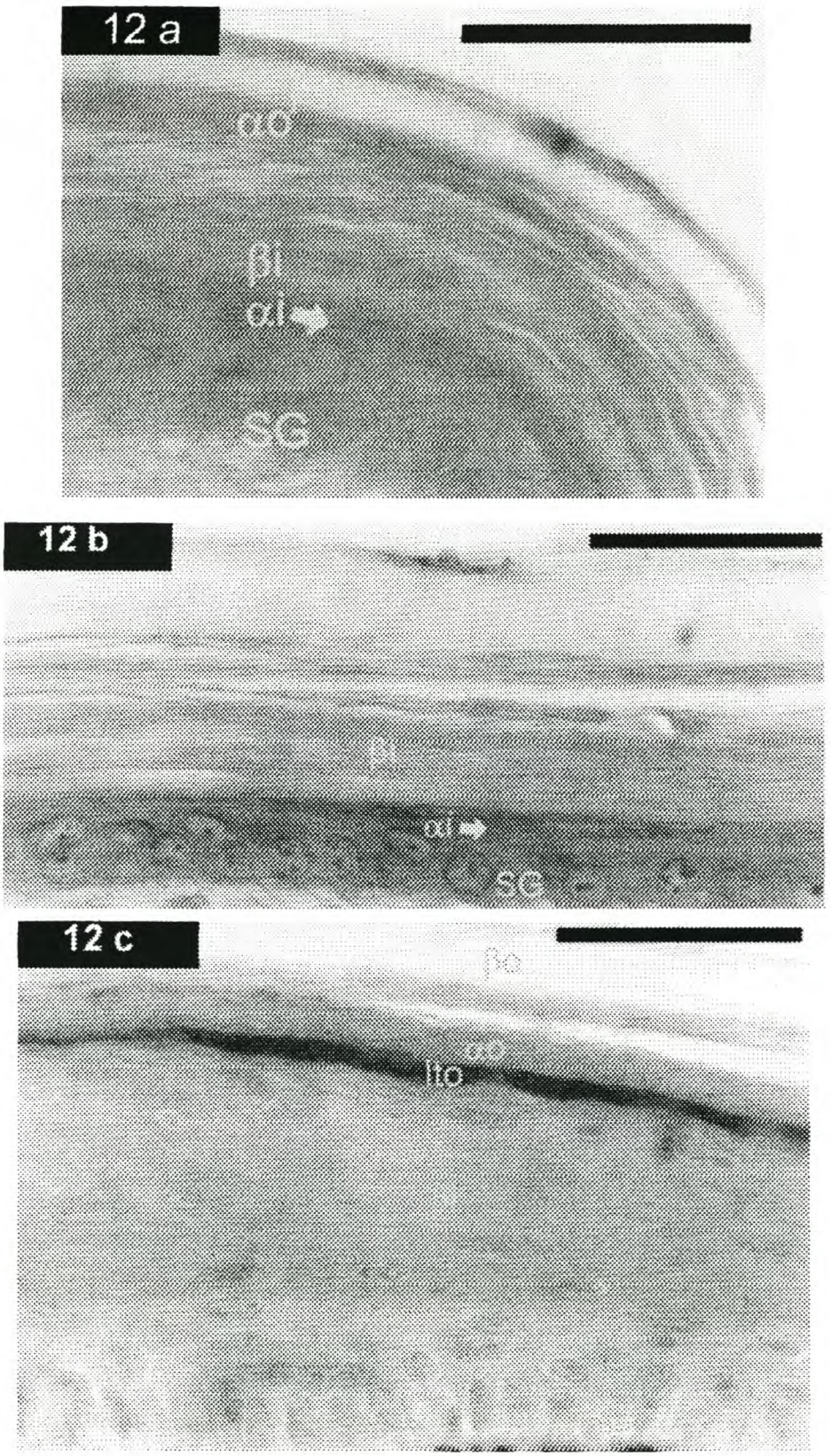


Figure 12 Unspecialized skin scales, displaying skin-renewal stage four of: a) *P. microlepidotus*; b) *C .cordylus;* and c) *P. capensis*. , scale bar = 1000 μm.

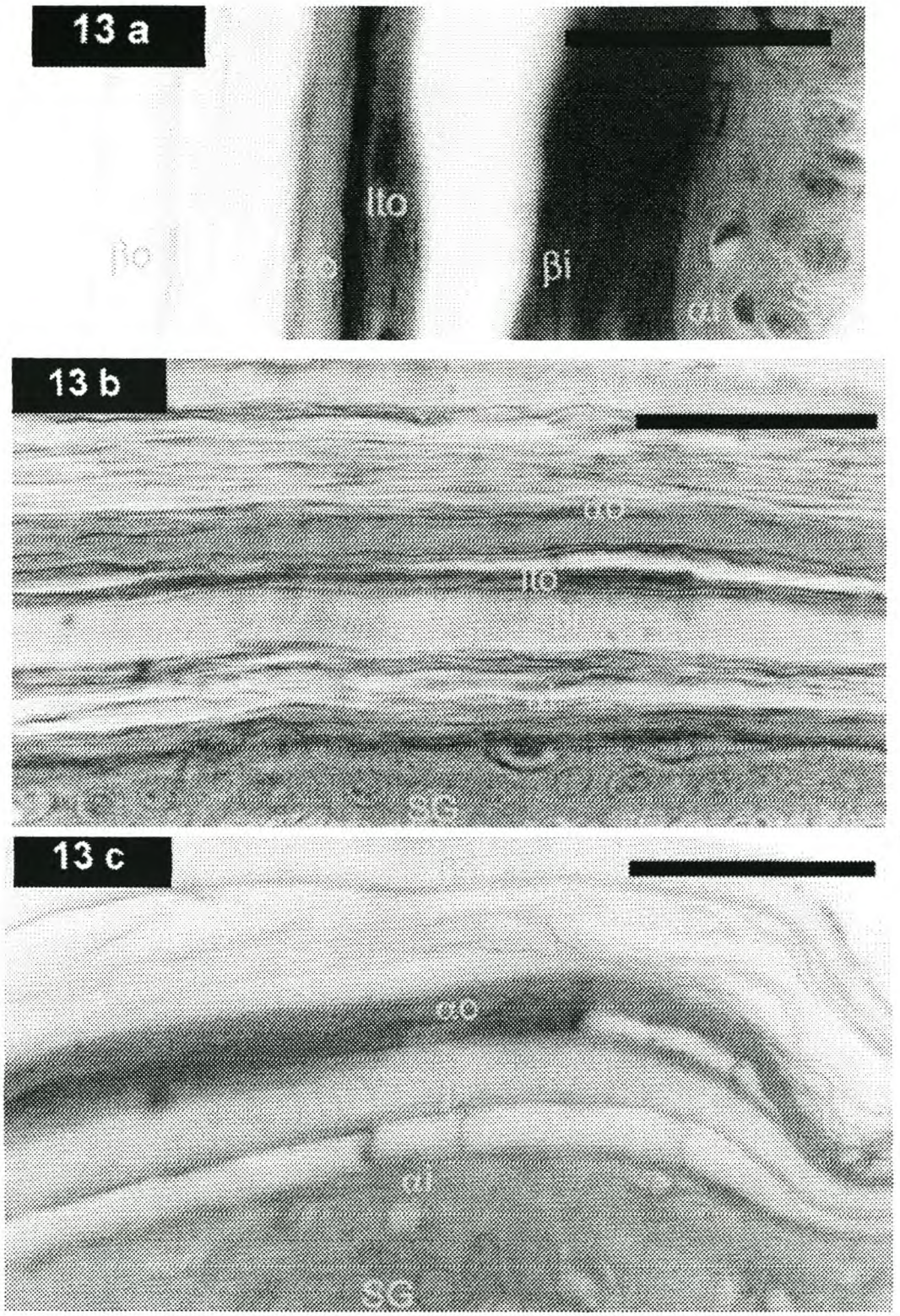


Figure 13 Unspecialized skin scales, displaying skin-renewal stage five of: a) *P. microlepidotus*; b) *C. cordylus* and c) *P. capensis*. scale bar = 1000 μm.

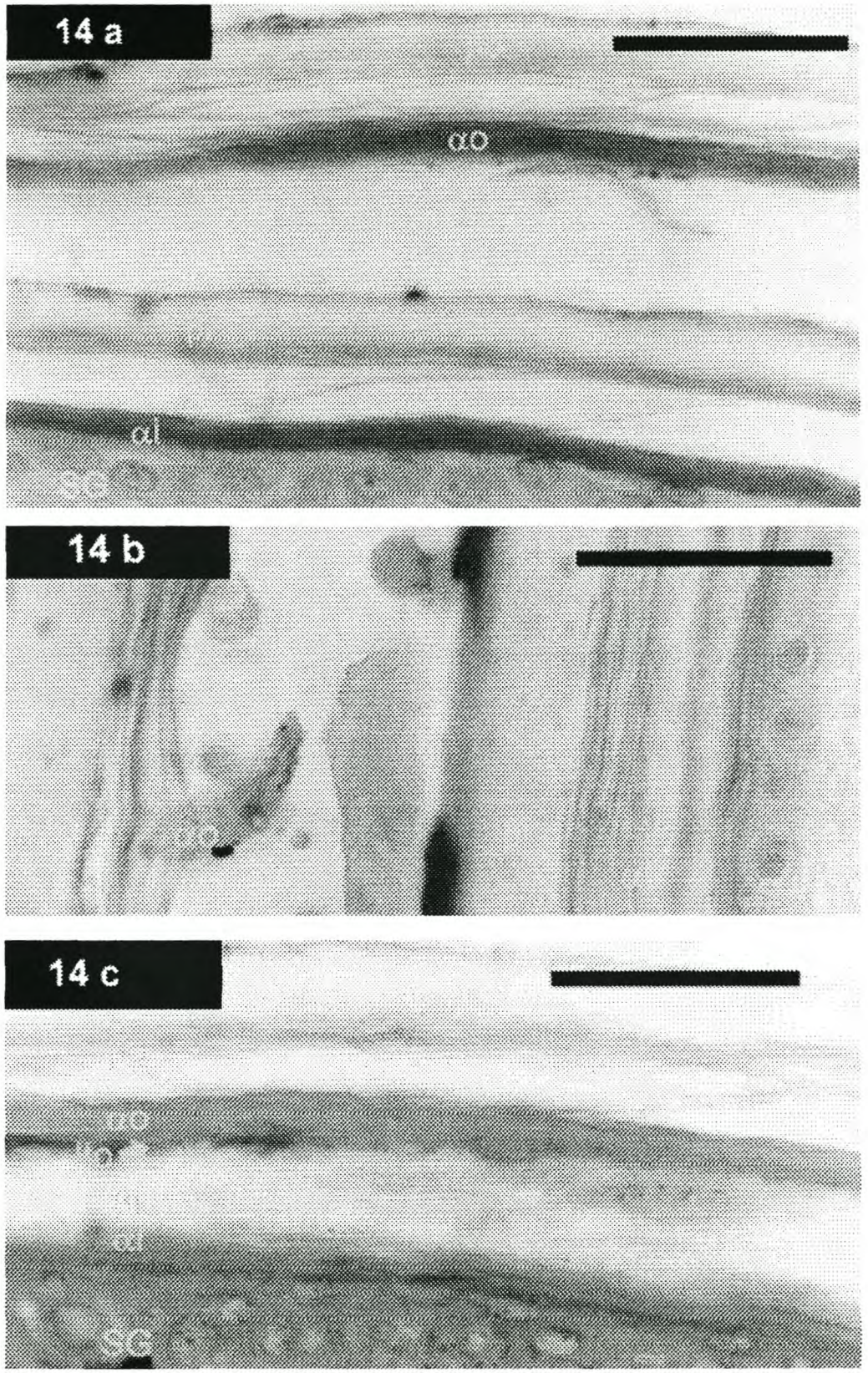


Figure 14 Unspecialized skin scales, displaying skin-renewal stage six of: a) *P. microlepidotus*; b) *C. cordylus* and c) *P. capensis*. scale bar = 1000 μm.

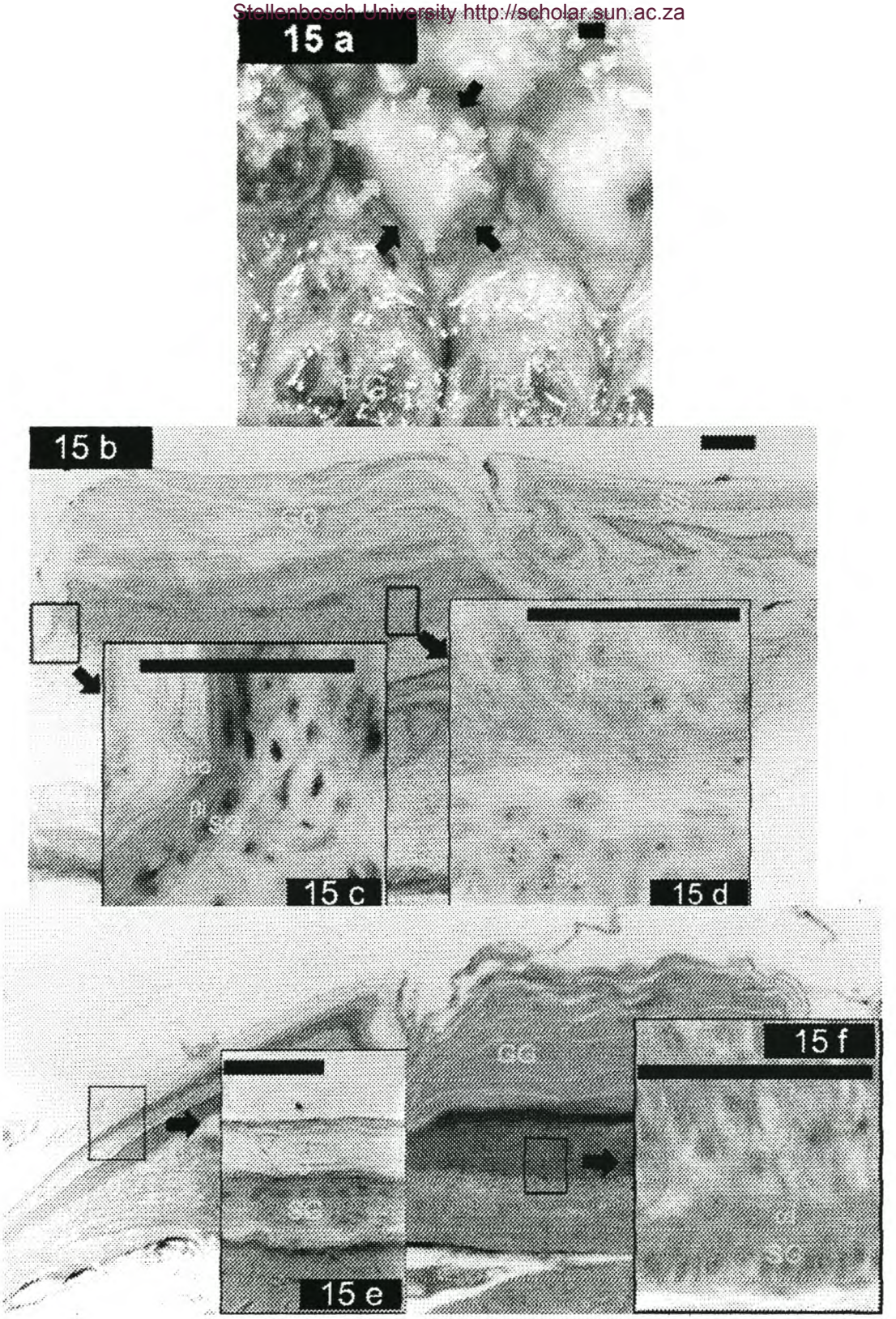


Figure 15, a) Stereozoom micrograph of underside of thigh of *P. capensis*, displaying differentiated (yellow arrows) and unspecialized (black arrows) skin; b) Generation gland and skin scale of *C. cordylus*; c) Peripheral unspecialized skin of the generation gland, stage three skin-renewal; d) Differentiated glandular region of the generation gland, also stage three skin-renewal; e) Unspecialized skin of the skin scale adjacent to the generation gland, PRS; f) Differentiated glandular region of the generation gland, stage four skin-renewal. scale bar = 1000 μm.

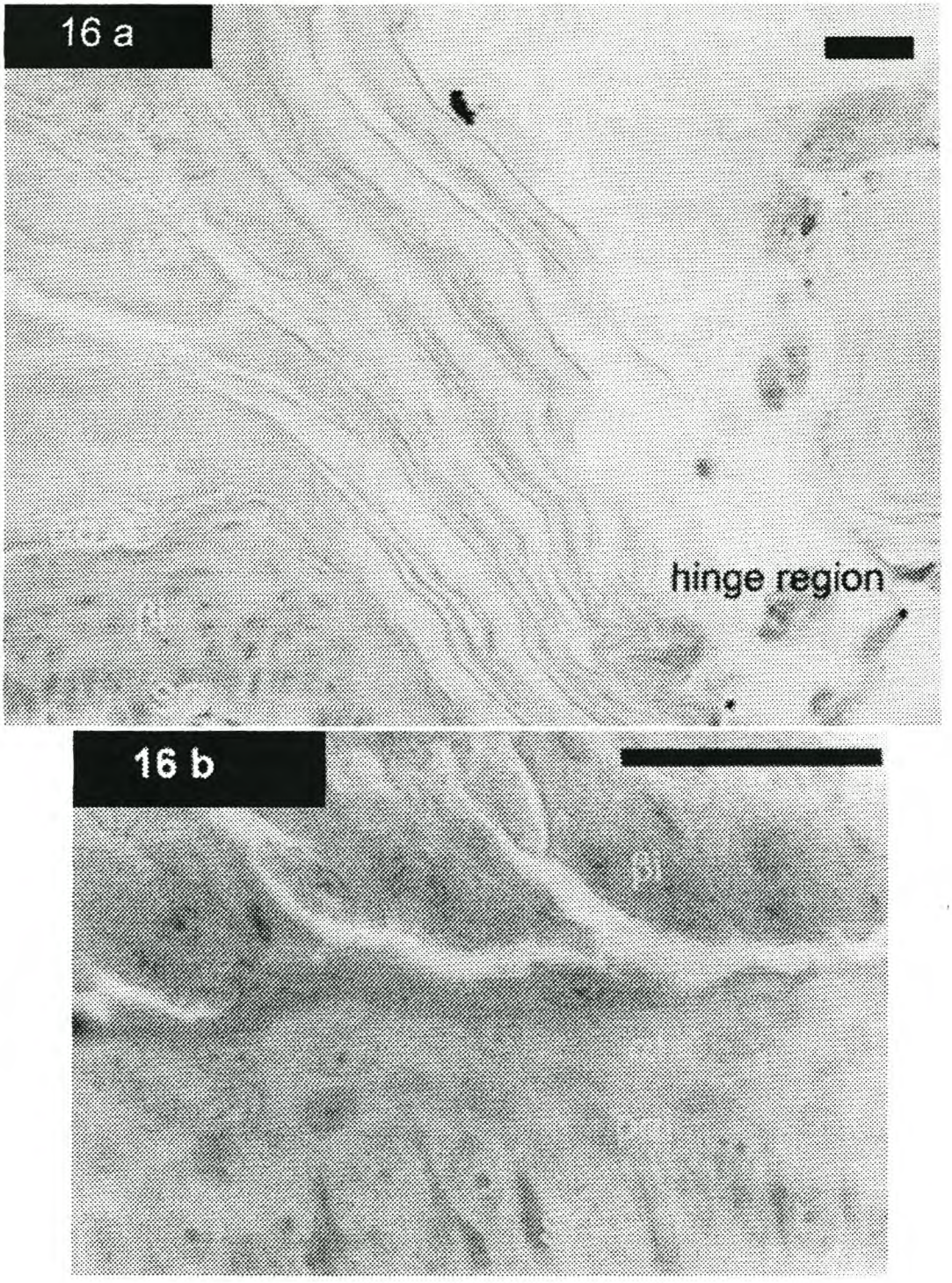
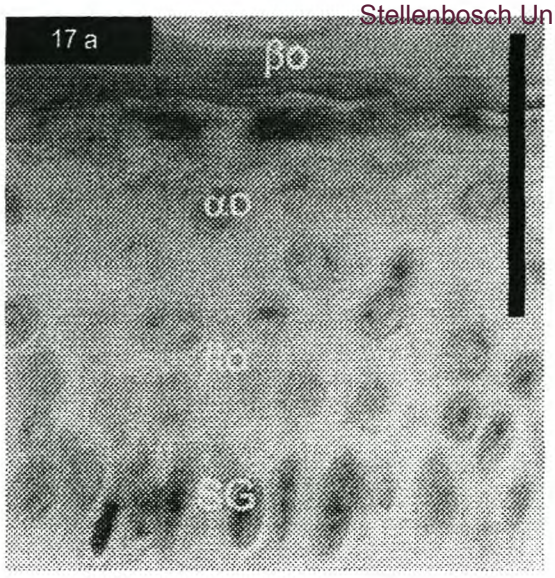
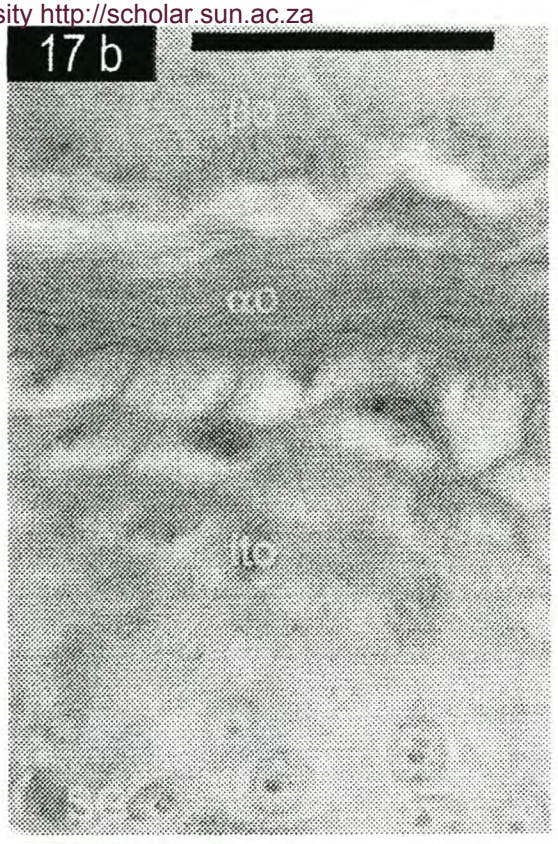
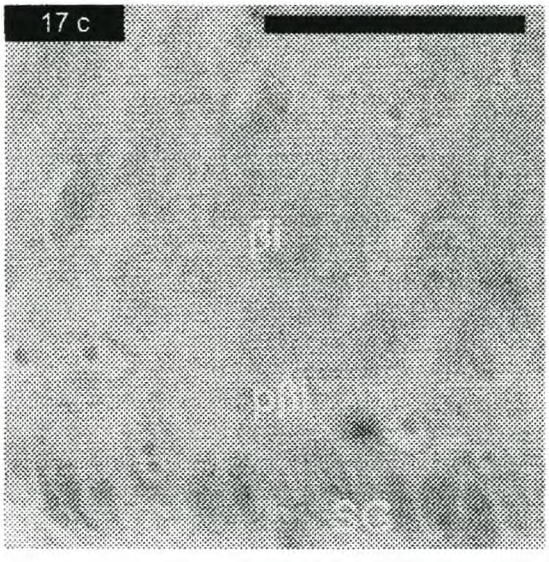
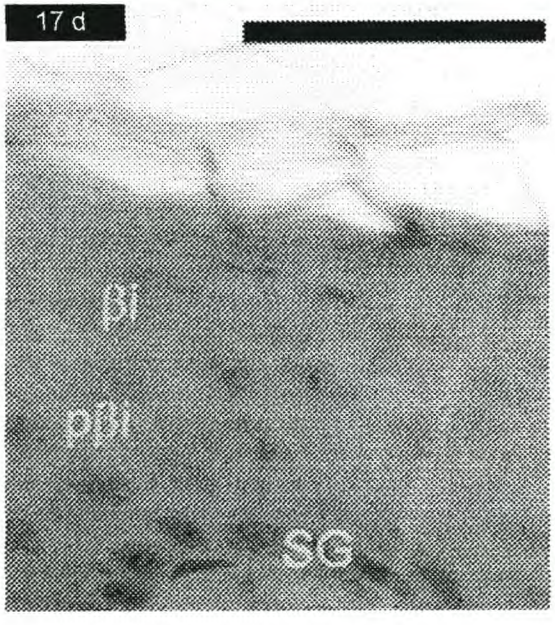


Figure 16, *C. cordylus* multi-layered generation gland a) showing multi-layered mantle attached at glandular material, yet free at hinge region and b) High magnification of the basal region showing renewal stage one histology. scale bar = 1000 μm.









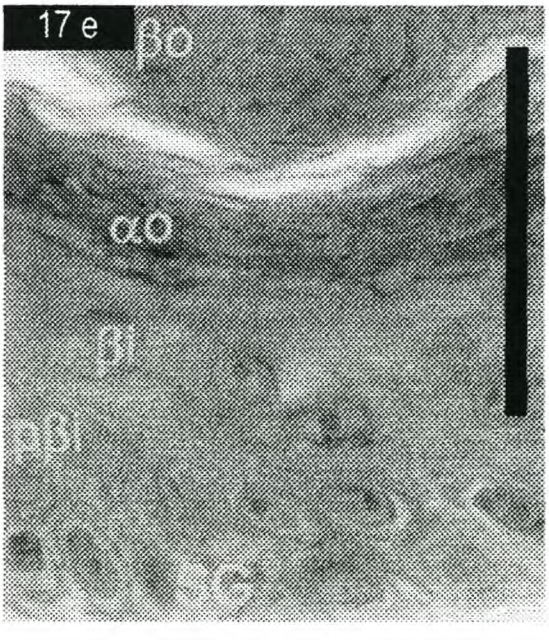


Figure 17, Basal region of cordylid generation glands, displaying various renewal stages: a) *P. capensis*, skin renewal stage two; b) *P. microlepidotus*, skin renewal stage two; c) *C. cordylus*, skin renewal stage three; d) *P. capensis*, skin renewal stage three; e) *P. microlepidotus*, skin renewal stage three. scale bar = 1000 μm.

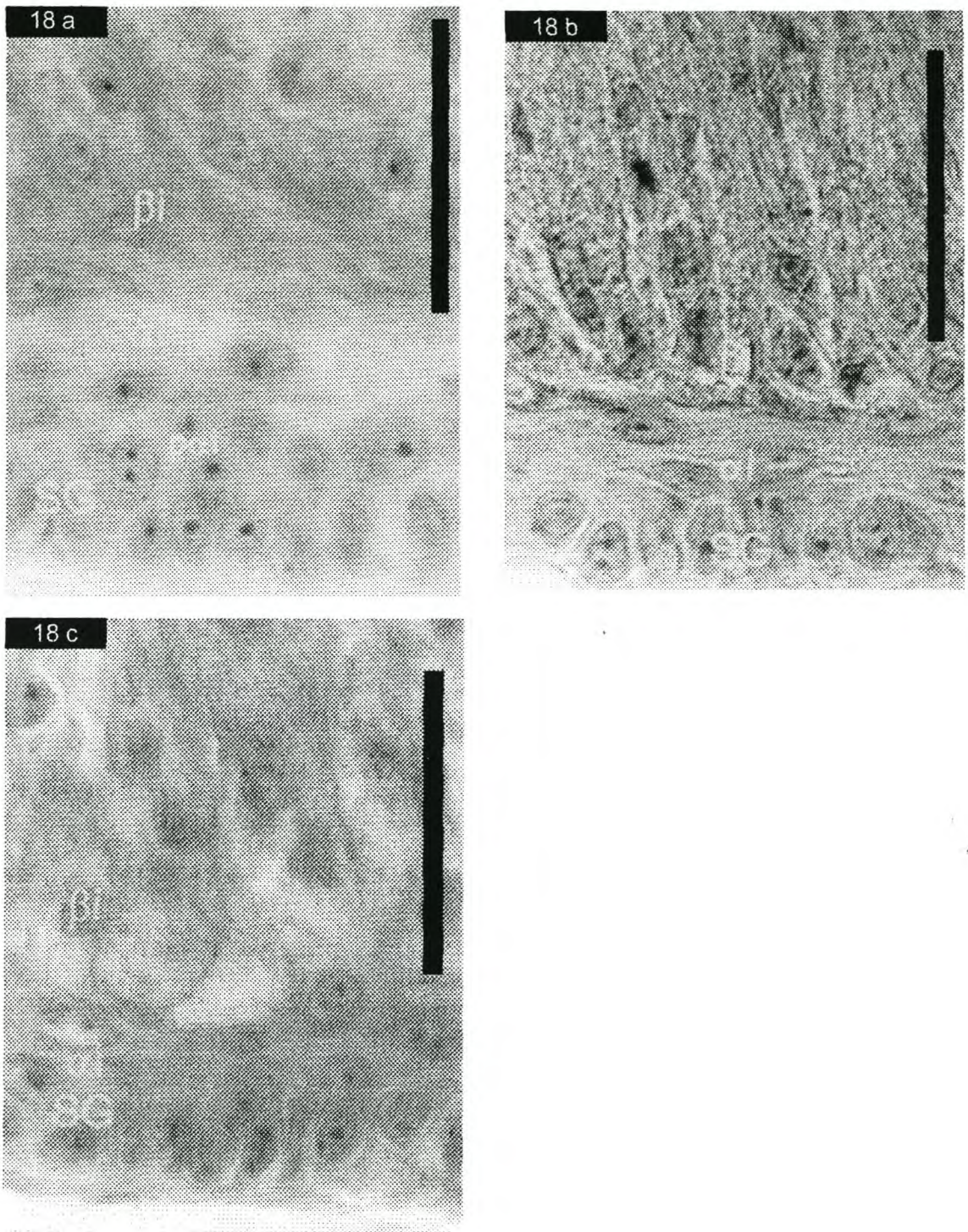


Figure 18, micrograph of basal region of cordylid generation gland displaying skin renewal stage four of: a) *C. cordylus*; b) *P. capensis* and c) *P. microlepidotus*. scale bar = 1000 μm.

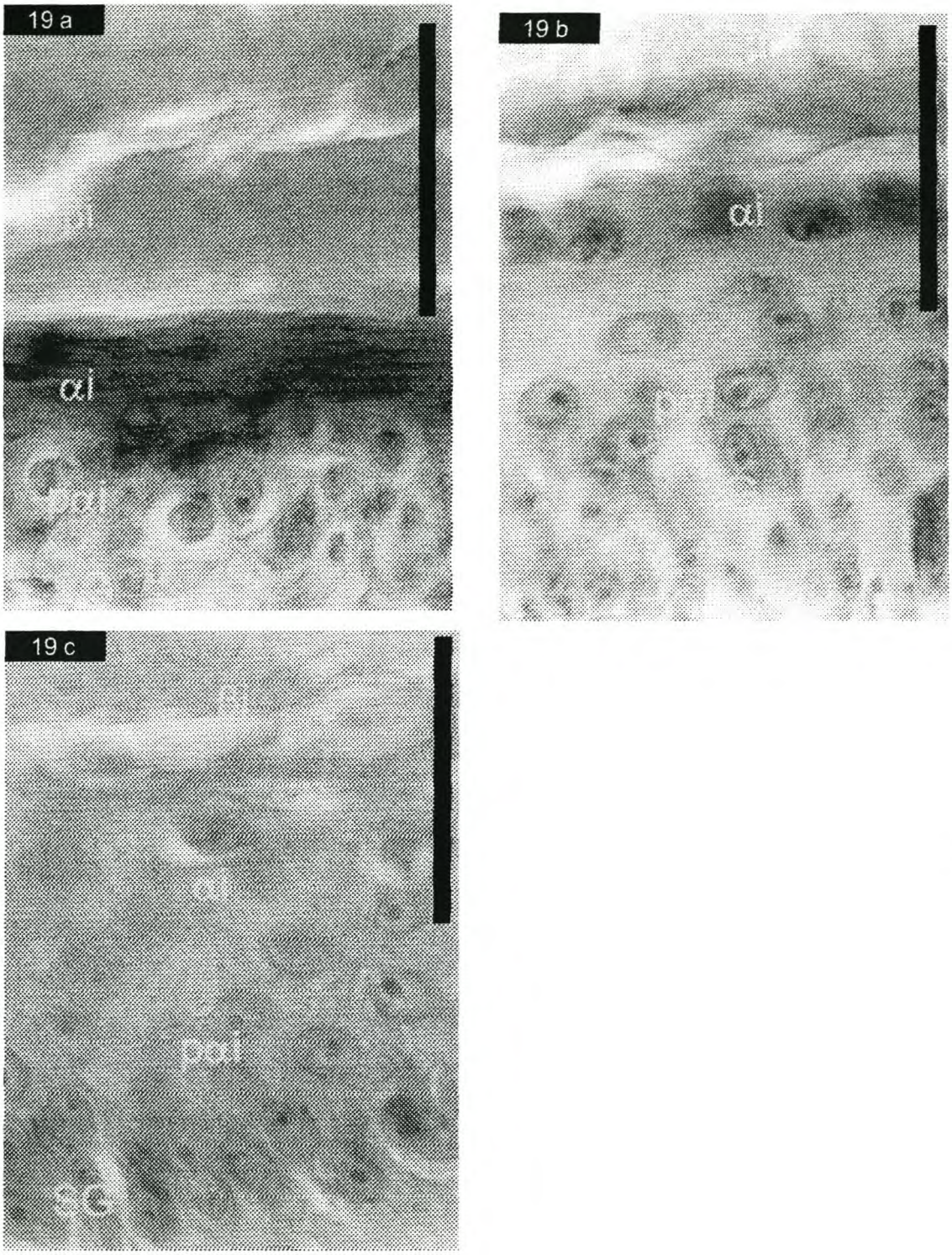


Figure 19, Basal region of cordylid generation gland displaying skin renewal stage five of a) *C. cordylus*, b) *P. capensis*, and c) *P. microlepidotus*. scale bar = 1000 μm.

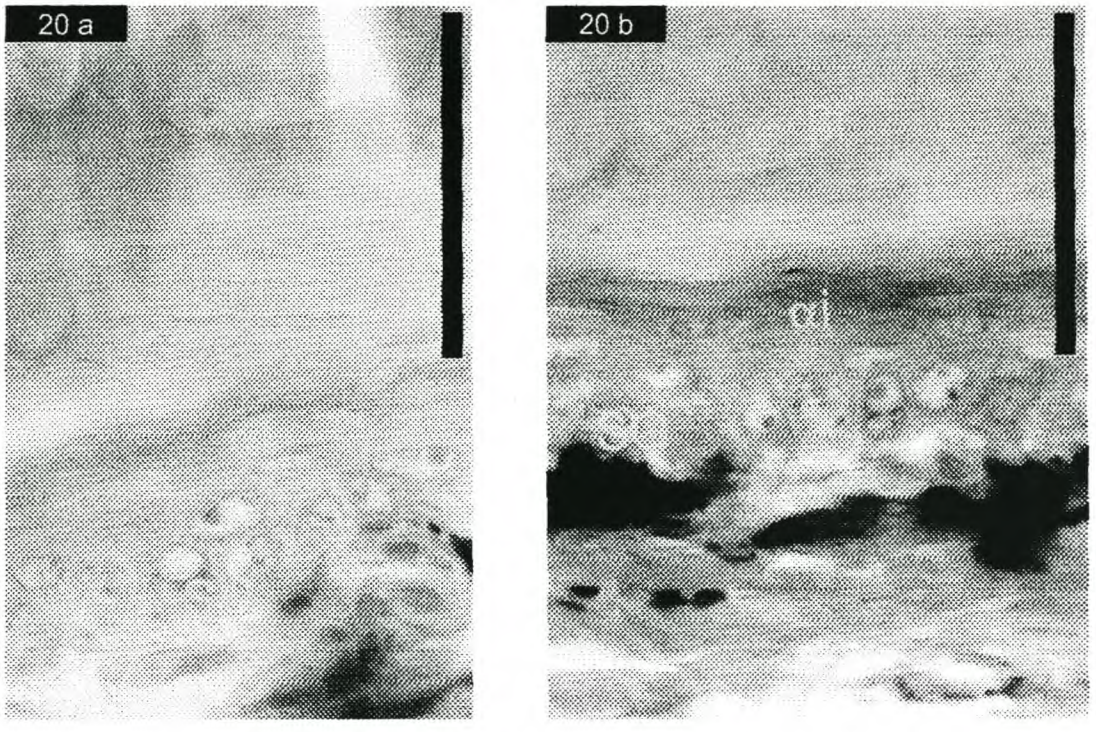


Figure 20. a) Basal region of generation gland displaying skin renewal stage six of a) *C. cordylus* and *P. capensis*. scale bar = 1000 μm.

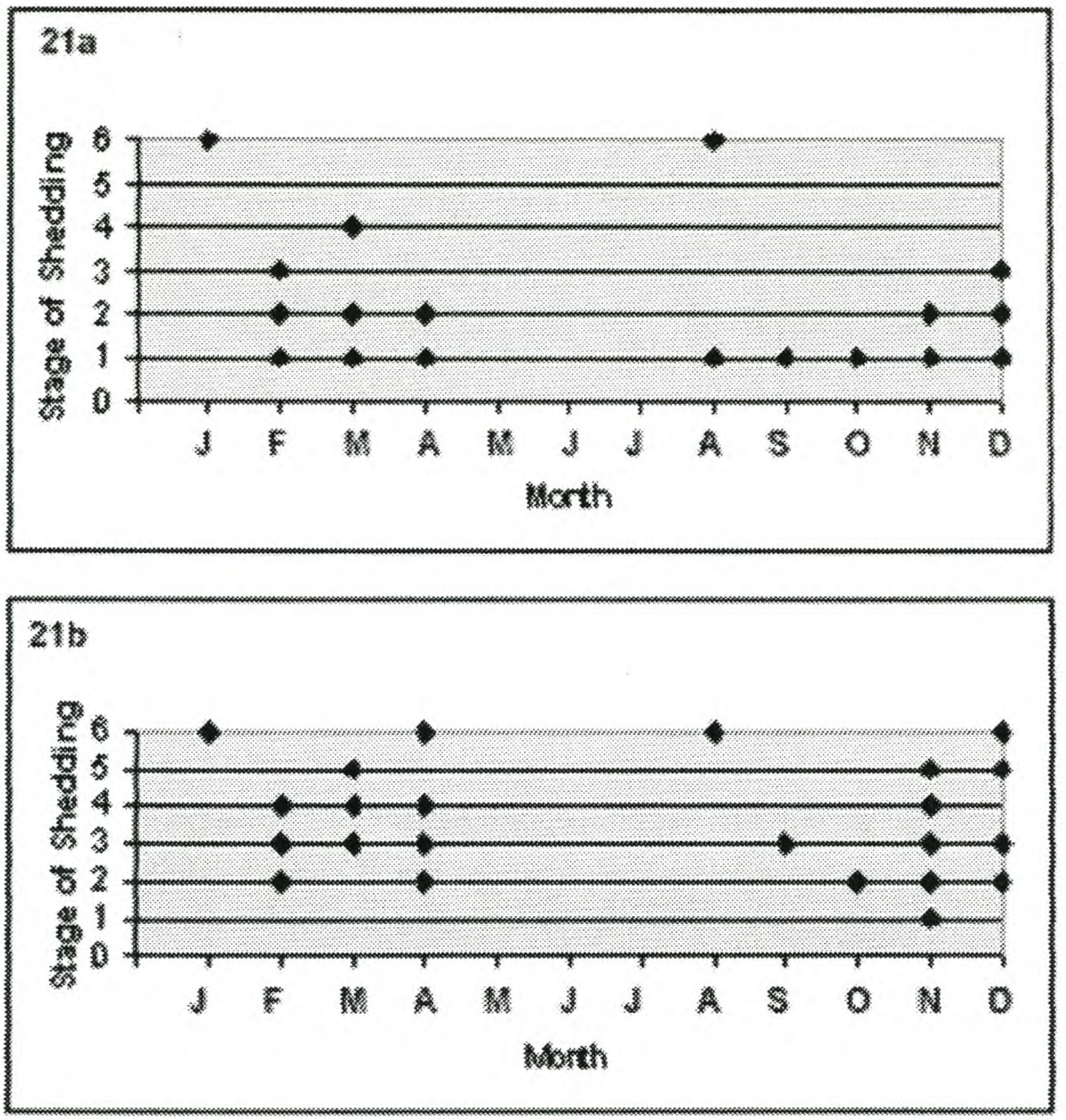


Figure 21. *P. capensis* shedding stages displayed at the time of year of collection, on all specimens' a) unspecialized skin scales and b) generation glands.

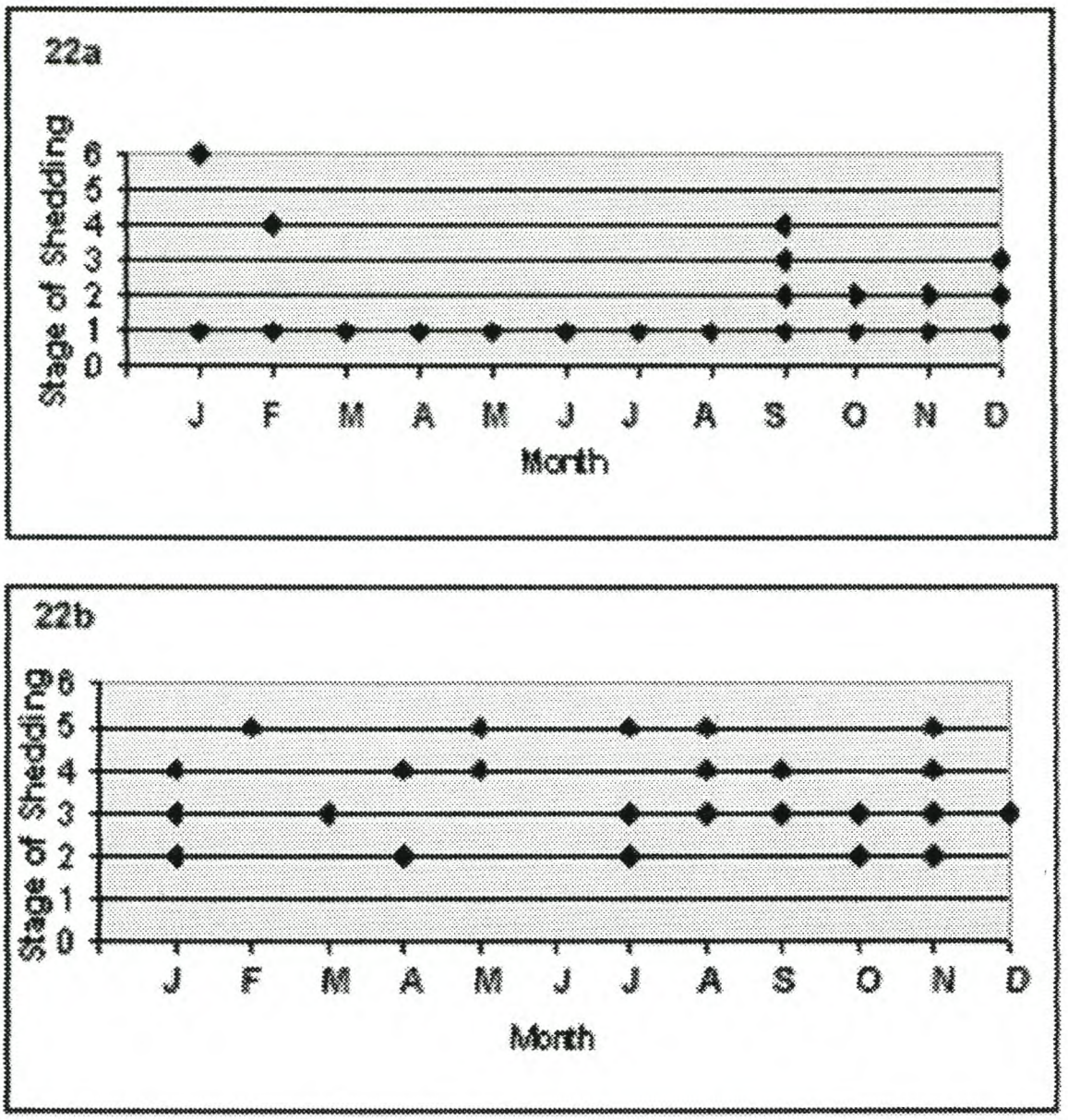


Figure 22. *C. cordylus* shedding stages displayed at the time of year of collection, on all specimens' a) unspecialized skin scales and b) generation glands.

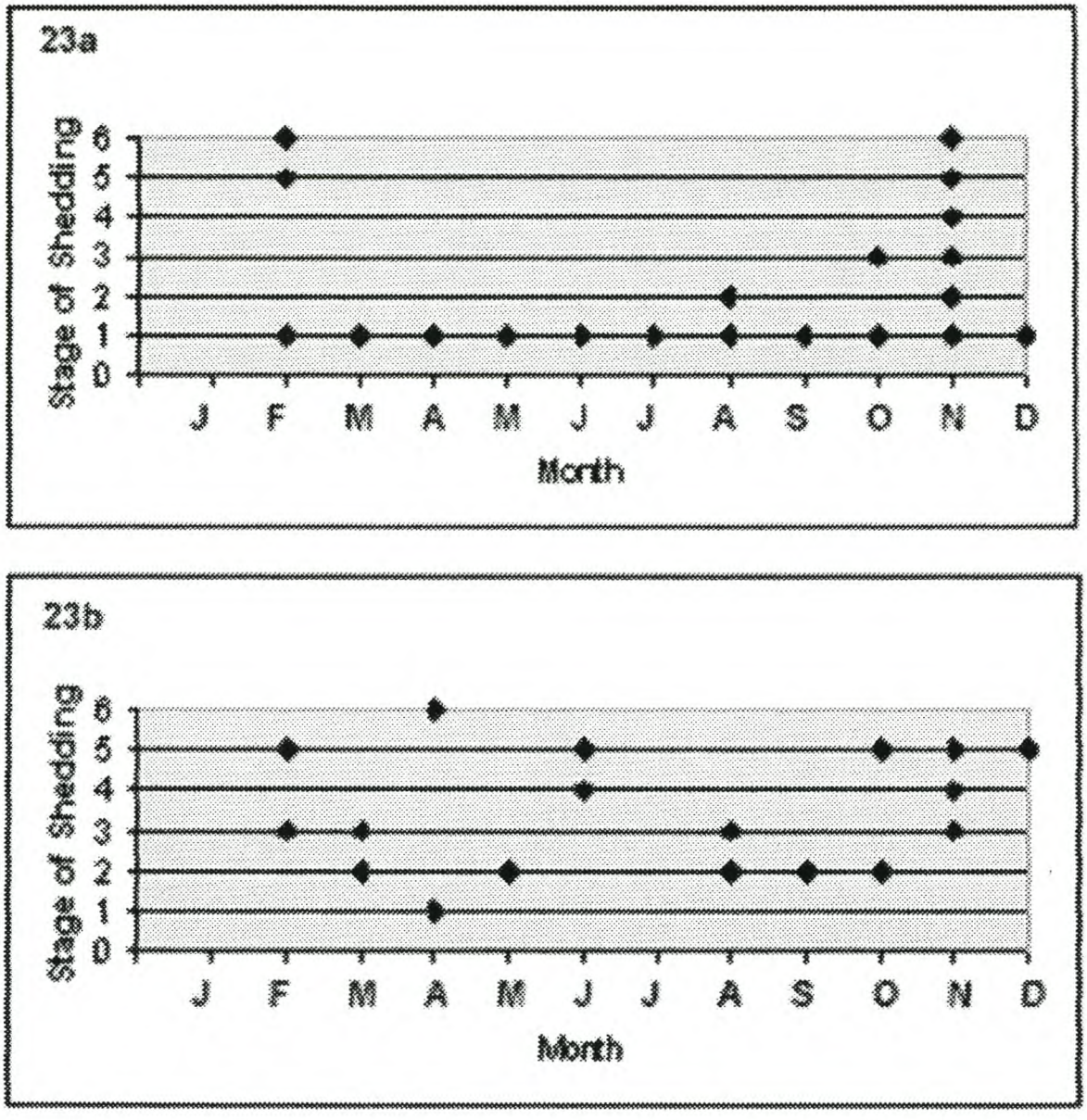


Figure 23. *P. microlepidotus* shedding stages displayed at the time of year of collection,on all specimens' a) unspecialized skin scales and b) generation glands.

FOUR

SEASONAL AND INTERSPECIFIC VARIATION IN PROLIFERATION RATES IN THE SKIN AND ASSOCIATED EPIDERMAL GLAND TYPES IN CORDYLID LIZARDS

4.1. INTRODUCTION

The squamate epidermis has a unique structure and method of renewal amongst amniotes (Maderson, 1970; Roth & Maderson, 1968). Epidermal renewal in reptiles is not a continuous process as in mammals and birds, but is divided into a rest, or non-proliferative phase and a renewal phase (Maderson, 1985). During the rest phase the epidermis is covered by an incomplete epidermal generation (EG) consisting of an oberhautchen/β-layer syncitium and an α-layer, and when the skin enters a renewal phase, it undergoes six renewal stages to complete this EG, and produce another incomplete EG below it (Maderson, 1966, 1985; Maderson et al., 1998). The process of skin shedding in reptiles is a synchronous occurrence over the whole body epidermis (Maderson, 1966). Epithelial renewal varies cyclically from high to low as the renewal cycle shifts from renewal phase to rest phase (Flaxman & Maderson, 1973). Current data for lizards indicates that the renewal phase lasts for approximately 14 days, and the rest phase varies in length, resulting in the duration for a complete shedding cycle, from rest phase to rest phase, being between 35 to 55 days (Maderson, 1985).

The epidermal renewal activity of the unspecialized skin scales of cordylids displayed seasonal variation. During cool, winter months, cordylids displayed a period of several months where no renewal activity was observed over the unspecialized epidermis, and renewal took place in the warmer months (Chapter Three). The generation glands were shown to shed in a manner asynchronous with that of surrounding unspecialized skin scales, and displayed no apparent seasonal behaviour. The activity of the skin glands was shown by histology, and this only showed the histological structures of the cells, yet gave no indication of their state of mitotic activity. It remains a possibility that the glands may be asynchronous with unspecialized skin, yet when the unspecialized skin enters a period of non-

proliferation over winter months, the generation glands also become dormant, displaying a histological picture of arrested development.

The objectives of this experiment are to use $3H^{+}$thymidine along with autoradiography and scintillation counts to test the following hypotheses:

(1) Variation will be seen in epidermal renewal rate between species investigated as shown by differences in the mitotic activity in the SG of the unspecialized skin scales and generation glands.

(2) Variation will be seen in epidermal renewal rates between unspecialized skin scales, and specialized generation glands, in all species investigated as shown by differences in the mitotic activity in the SG.

(3) Variation will be seen to show seasonal variation in epidermal renewal rates of the SG in two areas. These areas are seasonal variations in activity in unspecialized skin scales of representative species displaying the different generation gland types, and seasonal variations between the gland types themselves.

(4) A secondary objective of this experiment was to show, using radioactive labelling and relative mitotic activity, the feature of asynchrony between generation glands and unspecialized skin scales in cordylid lizards

4.2 MATERIALS AND METHODS

Specimens

Biopsies were taken from *Pseudocordylus capensis* (single-layer stacked gland), *Cordylus cordylus* (multi-layer stacked gland) and *P. microlepidotus* (multi-layer pit gland). Adult males of each species were collected from areas in the Western Cape of South Africa. *P. microlepidotus* were collected from Gansbaai, on Byeneskop (34° 34' S; 19° 21' E). *P. capensis* were collected from the Cedarberg (32° 22' S; 19° 02'E), and *C cordylus* were collected from Joostenberg (33° 50' S; 18° 52' E). All were housed individually in glass terrariums for six months between December 1999 and June 2000, and fed twice weekly on mealworms (Tenebrionidae), and fresh water was available at all times.

Seasonal biopsies: Biopsy removal:

Samples were removed once in summer (December) in the period where there is shedding activity of the SG of the unspecialized skin (active renewal period)

and once in winter (June) in the period when there is no shedding activity of the SG of the unspecialized skin (non-proliferative rest period).

Biopsies were removed from the postero-ventral area of the left thigh, and included femoral glands, generation glands and normal skin scales. The lizards were rendered immobile by cold (placed in a freezer until they were insensitive to touch and could not right themselves when they were placed on their backs (Clifford, 1984). The site for biopsy removal was cleaned with 70% ethyl alcohol. Biopsies were removed with scissors, and placed in the culture medium.

Culture procedure

All biopsies were incubated in 1 ml culture medium (RPMI-HEPES, containing 10% heat inactivated FCS (foetal calf serum) and 100 units per millilitre of penicillin, streptomycin and Fungizone) with 6 μCi $3H^+$-thymidine for 24 hours at 30° C. The biopsies were then washed three times with 70% ethyl alcohol and left in 70% alcohol overnight to extract free thymidine. Each biopsy was divided in two, using one half for autoradiography, while the other half was separated into individual scales to obtain relative radioactivity levels, using scintillation counting.

Autoradiography

Autoradiography, using tritiated thymidine, will mark cells that actively mitotic. Since SG cells that are actively producing a new skin generation will be mitotically active, they will take up $3H^+$-thymidine (Jørgensen & Levi, 1975). Histological examination of labelled material will indicate areas within the skin that are actively mitotic by the presence of labelled nuclei.

Following incubation, half of each biopsy was taken for histology and autoradiography, to compare skin and generation glands. These samples were fixed in cold (4 °C) modified Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde). Material was subjected to routine histological procedure, using paraffin wax (Paraplast: Sherwood Medical Co.) as embedding medium. Sections were made on a Reichert-Jung rotary microtome at 10µ, and mounted on glass microscope slides.

Ilford LM emulsion was used throughout the experiment. Under darkroom conditions, the emulsion was melted at 43 °C in a water bath. Slides were dipped in this emulsion and air-dried. They were kept in the dark at 4 °C, in sealed lightproof

boxes with silica gel to remove free moisture. The exposure time was four weeks, after which the slides were developed using Ilford Phenisol High Contrast Film Developer, diluted 1:4. They were immersed in a stop solution of 0.5% w/v acetic acid and then fixed in a solution of 30% w/v sodium thiosulphate. Slides were lightly stained with haematoxylin and eosin and mounted with HyperMount. Autoradiograms were viewed under oil immersion and nuclei containing more than 5 silver grains were counted per scale type.

Relative mitotic activity determination

Femoral gland (FG), generation gland (GG) and skin (SS) biopsies were placed in individual microcentrifugue tubes. 100 µl 1M NaOH was added to each tube to hydrolyze the samples. Hydrolysates were counted on a UV spectrophotometer at 260 nm, and were then added to 4 ml of Scintillation fluid and were counted on a Powerwave$_X$ spectrophotometer (Bio-Tech Instruments Inc.). The different scale types (femoral glands, generation glands and unspecialized skin scales) that were hydrolysed for the relative mitotic activity information differed in size. To remove the effect of size, scintillation counts (radioactivity) were expressed as a function of the fraction of the hydrolysate that absorbs light at Optical Density (OD) 260 nm. 260 nm is the wavelength that is absorbed primarily by DNA.

In comparison to the cellular labelling counts, where only minimum values can be determined, the scintillation counts/OD260 involves ALL radioactive counts. Removal of the blanko value subtracts the possible radioactivity levels of the NaOH and the scintillation fluid from each scales radioactivity count, but leaves behind in the value, the cumulative background counts and all radioactivity absorbed/incorporated into the dermis, and any other tissues present. Thus, the data represent values that may be higher than would have been seen if only epidermis had been hydrolysed.

Statistical Procedures and Calculation Methods

One-Way Analysis of Variance (ANOVA) and Student t-tests were used to determine variation between species, season and between skin and generation glands. All statistical procedures were done using the SigmaStat (Jandel Scientific) statistical package.

4.3 RESULTS

4.3.1: DECEMBER: ACTIVE PERIOD OF RENEWAL ACTIVITY

The unspecialized skin of the biopsies removed from specimens of *C. cordylus* and *P. capensis* in this month (summer) was seen to be in perfect rest phase (PRS), and skin shedding appeared to be completed (Table 1). Biopsies removed from specimens of *P. microlepidotus* were seen to show either stage four or six of the skin shedding cycle.

4.3.1.1: NUMBER OF LABELLED NUCLEI OF SG (FIGURE 24A)

These were counted on the 10µ sections from the autoradiography part of the experiment. Both *P. capensis* and *C. cordylus* showed no radioactive labelling in unspecialized skin scales (Figure 26 a & b). Unspecialized skin in *P. microlepidotus* exhibited advanced stages of epidermal renewal, and radioactive labelling occurred, indicating some mitotic activity (Figure 26 c).

Comparison of skin scales with generation glands in summer: Comparison of mitotic activity of the unspecialized skin with generation glands, within each representative species showed statistically significant differences ($p < 0.0001$) in *C. cordylus* (multi-layered stacked gland) and *P. capensis* (single-layer stacked gland), but no significant difference ($p > 0.05$) in *P. microlepidotus* (multi-layer pit gland). *P. capensis* and *C. cordylus* had the high mitotic activity levels in generation glands at the time the biopsies were removed, and no activity in the skin scales. High standard deviation in the generation gland mitotic activity mean in *P. microlepidotus* resulted in comparative levels that were too close for significant differences. Thus, the stacked generation gland types showed significantly higher labelling than did the unspecialized skin in the representative species. Pit glands undergoing renewal showed no significant difference from unspecialized skin scales likewise undergoing renewal in *P. microlepidotus*.

Comparison of unspecialized skin between species in summer: Comparison of mitotic activity of the SG with regard only to skin shedding activity amongst the three species showed that both *C. cordylus* and *P. capensis* showed statistically significant differences ($p < 0.05$) to *P. microlepidotus*, but did not differ significantly from each other ($p > 0.05$). Skin scales in *C. cordylus* and *P. capensis* were staged (Table 1) at PRS, showing no skin shedding renewal activity on a histological level,

and no radioactive labelling in the SG below the epidermal layers. *P. microlepidotus* displayed both stages four and six of the renewal stages and moderate labelling by radioactive $3H^+$-thymidine and so differed from the other species.

Comparison of generation gland-types between species in summer: Comparison of mitotic activity of the SG with regard only to generation gland renewal activity among the three species showed that both *C. cordylus* and *P. microlepidotus* differ with a statistically significant figure ($p < 0.05$) from *P. capensis*, but not from each other ($p > 0.05$). *P. capensis* displayed the highest levels of radioactive labelling of the three species. Large standard deviation (SD) in *C. cordylus* generation glands caused the lack of statistical difference when this species was compared to *P. microlepidotus* generation glands, the species displaying the lowest mean of radioactive labelling.

4.3.1.2: RELATIVE MITOTIC ACTIVITY (FIGURE 25A)

Comparison of skin scales with generation glands in summer: When comparing unspecialized skin scales with generation glands within the representative species, variation in relative mitotic activity was found to differ significantly ($p < 0.05$) in both *P. capensis* and *P. microlepidotus*, but not in *C. cordylus.* Thus, the multi-layer stacked gland was the only generation gland type to show no significant difference when compared to surrounding unspecialized skin scales, with regard to the relative mitotic activity of the scale.

Comparison of unspecialized skin between species in summer: Unspecialized skin of *C. cordylus* had the highest mean relative activity in this period and differed from both *P. capensis* and *P. microlepidotus* with a statistically significant difference ($p < 0.05$). As both *P. capensis* and *P. microlepidotus* had low relative mitotic activities in this period, they did not differ from each other significantly ($p > 0.05$).

Comparison of generation gland-types between species in summer: All three species displayed different relative activity levels, but due to the closeness of these levels, only the highest (*C.* cordylus) differed significantly ($p < 0.05$) from the lowest (*P. capensis*).

4.3.2: JUNE: DORMANT PERIOD OF RENEWAL ACTIVITY

C. cordylus and *P. microlepidotus* specimens all displayed non-proliferation, displaying perfect rest stage (PRS), as were most *P. capensis* specimens. Two specimens of *P. capensis* were in stage two and one specimen of *P. microlepidotus* showed relatively high counts of labelled nuclei in the SG, although it was displaying PRS (Figure 27a).

4.3.2.1: NUMBER OF LABELLED NUCLEI OF SG (FIGURE 24B)

All species showed an increase in radioactive labelling in biopsies removed in this sample period. Labelled nuclei in the unspecialized skin of all species appeared to be concentrated near the hinge area, although some nuclei were visible in the SG of the scale itself (Figures 27b & c). *P. capensis* specimens that showed PRS all had very low labelling counts in the normal skin, as did *C. cordylus* specimens. Generation glands of all species displayed high labelling counts in this sample period, with *P. microlepidotus* displaying the highest mean (38 nuclei per 10μ section).

Comparison of skin scales with generation glands in winter: Generation gland mitotic activity was much higher than that of skin scales, and in all species, these scale types differed significantly from each other ($p < 0.05$). Differences between scale types' labelling was so marked in *C. cordylus* and *P. microlepidotus* that these differences were very significant ($p < 0.0001$).

Comparison of unspecialized skin between species in winter: C. cordylus showed extremely low labelling when compared to the other two species, and differed significantly from both ($p < 0.05$). *P. capensis* and *P. microlepidotus* had different mean mitotic activity in unspecialized skin scales, but large SD in both meant these were not significantly different.

Comparison of generation gland-types between species in winter: All three species showed higher counts of radioactivity than was displayed in December, but it was *P. microlepidotus* that made the most pronounced change, going from the species with the lowest radioactivity count in December to the species with the highest count in June. *P. microlepidotus* differed significantly ($p < 0.05$) from both other species. While *C. cordylus* and *P. capensis* had different mean cellular activities, large SD in both caused there to be no statistically significant difference.

4.3.2.2: RELATIVE MITOTIC ACTIVITY (FIGURE 25B)

Scintillation counts for skin scales from the June sampling period were so low that upon subtraction of blanko values, *P. capensis* unspecialized skin scale hydrolysates yielded negative radioactive counts. This made data analysis for this scale type in this species and sampling period impossible, and only generation glands in this species can be compared with other species.

Comparison of skin scales with generation glands in winter: There were no statistically significant differences ($p > 0.05$) between skin scales or generation glands in either *C. cordylus* or *P. microlepidotus*. In both species, the means were very close together, and there was large SD in both skin scale derivatives (unspecialized skin scales and generation glands) in both species.

Comparison of unspecialized skin between species in winter: The only possible comparison was that between *C. cordylus* and *P. microlepidotus*, where no statistically significant difference ($p > 0.05$) was visible. *P. microlepidotus*, which had a mean relative mitotic activity that was almost five times the amount of that of *C. cordylus*, also showed SD that was almost eight times as large as that of *C. cordylus*.

Comparison of generation gland-types between species in winter: Large SD in *C. cordylus* generation glands caused there to be no statistically significant difference ($p > 0.05$) between generation glands of this species, and those of *P. capensis*. Both of these species differed significantly ($p < 0.05$) from *P. microlepidotus* which had generation glands that showed the highest relative mitotic activity of any skin scale derivative of any species during the whole experiment.

4.3.3: COMPARISON BETWEEN SEASONS

4.3.3.1: MITOTIC ACTIVITY OF SG

When comparing the unspecialized skin of each species between sampling periods, with regard to cellular labelling counts, all species were found to differ significantly ($p < 0.05$) between seasons. The difference was in the opposite direction to that predicted, as all species displayed higher levels of radioactive nuclei in the seasonal period when the skin was theorized to be inactive.

The same situation was seen in the generation glands, where much higher labelling was seen in the samples taken in June. All comparisons within species between the different seasons were statistically significantly different ($p < 0.05$).

4.3.3.2: RELATIVE MITOTIC ACTIVITY (SCINTILLATION COUNTS/OD260)

C. cordylus unspecialized skin displayed a statistically significant difference between sampling periods ($p < 0.05$), with higher levels of relative mitotic activity in December (active epidermal renewal period), even though the skin displayed PRS in both sampling periods. *P. capensis* yielded negative results in the June sampling period and therefore this species' unspecialized skin cannot be compared between seasons. *P. microlepidotus'* unspecialized skin displayed no statistically significant difference between seasons, although this is likely to be due to high SD in June.

When comparing generation gland-types between seasons in the representative species, it was seen that both *P. capensis* and *C. cordylus* displayed statistically significant differences in relative mitotic activity between sampling periods ($p < 0.05$). Both species had much higher activity figures in December (active epidermal renewal period – warm months) than in June (dormant epidermal renewal period – cool months), the same situation as seen in the unspecialized skin scales. *P. microlepidotus* displayed no statistically significant difference, even though the mean figure in June was more than double that displayed in December.

4.4. DISCUSSION

The secondary goal of this experiment was to show, using radioactive labelling and relative mitotic activity, the feature of asynchrony between generation glands and unspecialized skin scales in cordylid lizards. Statistically significant differences between these scale types were observed in all species, although conspicuous differences between the various species regarding when they showed these differences, raise many questions. Significant differences in mitotic activity provide good evidence to bolster the histological evidence that cordylid generation glands are asynchronous from the surrounding unspecialized skin scales.

The main goals of this experiment was to determine seasonal variation of the different generation gland types and their related unspecialized skin scales in the different species. When both *C. cordylus* and *P. capensis*' generation glands from the samples removed in December were compared with those removed in June,

statistically significant differences were noted with regard to relative mitotic activity. Both species' generation glands showed more activity in the active skin renewal period (December) than in the dormant skin renewal period (June).

P. microlepidotus did not show statistically significant differences when similar comparisons were made, although a large SD in June could account for this. Comparison of mean values for this species showed that there was more than double the relative mitotic activity in June than observed in December. The statistics program indicated that the large SD inferred that negative results should be interpreted carefully, and this clearly seems to be the case.

Cellular radioactive labelling showed that generation gland types were significantly different between seasons ($p < 0.05$) with all species displaying more labelled nuclei in the SG in the dormant period of epidermal renewal (June).

Thus, both methods of analyzing scale activity indicate seasonal variation, although contradicting each other in which season the most activity was located. Data accumulated from the relative mitotic activity data set seems to be more reliable, as it describes all the incorporated nuclei in the scale, whereas autoradiography only indicates incorporation in the top 1µ of the 10µ section, thus indicating a minimum value. The advantage of autoradiography is that it indicates exactly *where* the mitotic activity and incorporation took place.

All species' generation glands in both experiments were in stages of active shedding. Some specimens did not show any mitotic activity in the generation gland, even though they were histologically in the middle of shedding (Stage 3). This could account for some of the variation between seasons within particular generation gland types. This could be a form of arrested development, whereby the generation gland, while being out of synchrony with unspecialized skin scales, is still to a degree affected by whatever controls shedding in unspecialized skin. Thus, when the skin completes epidermal renewal, and enters a rest phase, the generation gland's SG may either slow production of new cells, or cease production entirely. It is also possible that this could simply be an extremely short period between production of different skin layers, and samples just happened to be taken in that time.

When comparing radioactive labelling of unspecialized skin, seasonal variation was displayed in both *P. capensis* and *C. cordylus* but no significant

differences were found for seasonal variation in *P. microlepidotus*. All species displayed higher radioactive nuclei in the June sample, when skin was thought to be dormant (Figures 21a, 22a & 23a). When the relative mitotic activity was compared between seasons for unspecialized skin, only *C. cordylus* differed significantly between seasons. *P. capensis* data sets were negative in June, and thus data could not be compared. *P. microlepidotus* had very large SD in June (Figure 4.10) and this could have led to the lack of significance.

The seasonal variation of all three species was different, meaning that each species has a different pattern of epidermal activity. This was indicated previously in Chapter Three, where each species appeared to show epidermal renewal activity for varying lengths of time. There was no indication of how many shedding cycles took place in that period when the skin was seen to be actively shedding. Gekkonids have shown to display extended periods of rest phase during cool periods, and researchers have inferred that in cool periods these animals display low shedding frequency (Chiu & Maderson, 1980). Cordylids appear to behave in a similar manner, and since each of these species represent a different generation gland type, not only do generation glands display seasonal variation, but the skin which they are a part of, shows different patterns of activity. Thus, generation glands differ not only in their own seasonal activity, but also the different seasonal activity of the unspecialized skin that they are an intrinsic part of.

P. capensis (Single-layer stacked glands) show higher cellular labelling in the unspecialized skin than *C. cordylus (*multi-layered stacked glands). This, together with the increased amount of time spent shedding (Figures 21a, 22a & 23a), seems to indicate that *P. capensis* shows more renewal activity than *C. cordylus* and thus the single layer of glandular material on the *P. capensis* generation gland may be renewed more often, so the same amount of glandular material may be available for both species in a year. There are only indications of this phenomenon, and there must be further investigation to confirm this.

High relative activity counts and high cellular radioactivity labelling were found all at the same time as each other, in the sampling period that was designed to sample skin in the "dormant" period of epidermal renewal, or winter (June). A clear reason for this was that active shedding was not sampled in the unspecialized skin in summer (December) except in *P. microlepidotus*, which displayed skin

shedding renewal stages four and six. In the June samples, all species displayed PRS, except for two *P. capensis* specimens, which displayed shedding stage two. Why then were the skin scales and generation glands in the June sample found to display higher radioactive labelling and higher relative mitotic activity in all three species sampled, than those sampled in December?

Three possible answers are immediately obvious. The first is that the skin is about to begin shedding again and the OG must be completed, to begin stage two. Thus, the activity shown by the labelled nuclei/cells corresponds to initiation of proliferation within the SG. Stage two was observed in some *P. capensis* in June, lending some credence to this answer. However, according to the activity cycles described (Figures 21a, 22a & 23a), *P. microlepidotus* should only begin to start shedding in late spring, towards late September, or early October. *C. cordylus* is also not likely to be shedding so early in the year, although this is definitely a possibility. Stress, due to being kept in laboratory conditions for six months since the first biopsies were taken in December, could have initiated an early beginning of the proliferative phase. Maderson (pers. comm.) indicated that gekkonids captured and handled a few days before biopsy removal had all initiated renewal stage two.

The second possibility is due the area where the labelled cells occur in the SG. Labelling appears to be confined to the hinge region, inner scale surface (ISS) and the peripheral fifth of the outer scale surface. Since scales themselves are relatively inflexible, most flexing takes place in the hinge region (Maderson et al., 1998). α-layer cells that provide the barrier to cutaneous water loss (CWL) could be damaged in the hinge region, possibly by over-exerting the skin (Maderson pers. comm.). This would necessitate regeneration of the α-layer to repair the CWL barrier. Skin samples from December that are in PRS, have just completed shedding recently, so it would be unlikely that they would need repair. This corresponds to lower counts of the labelled cells at this time. Six months later, in June, increased labelling was noticed in the hinge region, even though the skin was still in PRS. Cracks or damage to the α-layer could be being repaired, and this would lead to mitotic activity, as new cells will be necessary to fulfil this need.

A third possibility is that testosterone levels could affect the shedding cycle of the skin. Testosterone levels show two peaks in the testicular cycle of a cordylid lizard, *Cordylus polyzonus* (Flemming, 1993). The species used in this thesis show

different patterns (Van Wyk, pers. comm.). *P. microlepidotus* has a small peak in testosterone level in spring, and a large peak in autumn. The situation is reversed for *C. cordylus* and *P. capensis*, with a large peak in spring and a small peak in autumn.

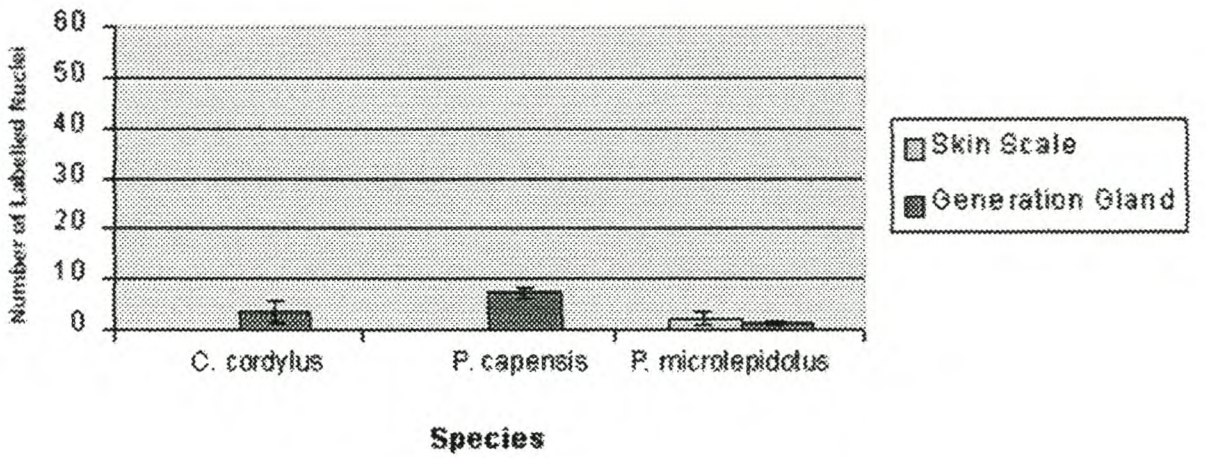
The first samples were taken in December, and this was chronologically placed after the high peak in testosterone levels in *C. cordylus* and *P. capensis* but before the high peak in *P. microlepidotus*. Assuming that the data, positively correlating androgen activity with generation gland activity, in gekkonids can be extrapolated to cordylids, the pattern revealed by December's results could be explained satisfactorily. Low labelling counts were displayed for both skin scales and generation glands in all three species. Significant difference ($p<0.05$) between *C. cordylus* and *P. capensis*, and no significant difference ($p>0.05$) between *C. cordylus* and *P. microlepidotus* can probably be attributed to high variation in labelling in *C. cordylus.*

The second sampling period was in June, and was chronologically placed shortly after the high peak in testosterone levels in *P. microlepidotus* and after the small peak in testosterone levels in *P. capensis* and *C. cordylus*. Significance was only shown between *P. microlepidotus* and *C. cordylus*, although very large standard deviations in *P. microlepidotus* affect data analysis. Examination only of mean values shows a high level of activity in *P. microlepidotus* while those found in *P. capensis* and *C. cordylus* are much lower.

Large standard deviations in both experiments can be attributed to several factors. Significantly wide variation in labelling counts between samples will lead to large standard deviations. This could be a result of the problems integral to the autographic method, where only the top 1µ will label on autoradiograms. The centrifugal proliferation gradient (Maderson et al., 1998) displayed on all reptilian scales, whereby only the mid-sagittal sections through the distal third of a scale can be used for staging, can result in incorrect staging, although this was corrected for by making serial sections through the whole scale. What can result are lower radioactive labelling on out-lying sections, increasing variation within the sample. *P. microlepidotus*, with multiple pits on a single generation gland scale, may display multiple centrifugal differentiation gradients within a single scale, although further research will be needed to verify this.

In conclusion, cordylid generation glands of all types display asynchrony with surrounding unspecialized skin scales. This has been proved from a histological viewpoint (Chapter Three) and now is further confirmed by autoradiography and relative mitotic activity analysis. Generation glands also display variation in seasonal activity patterns indicating they may be linked to seasonal activity patterns of the testicular cycle and may be controlled to some degree by the action of androgens.

24a



24b

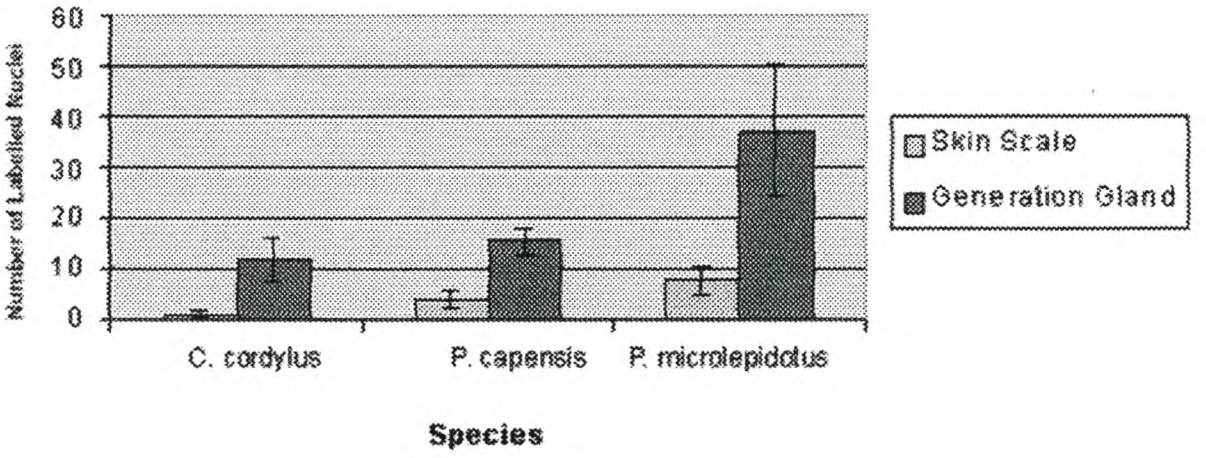


Figure 24. Results of labelled thymidine incorporation by the skin and generation glands of three cordylid species. Tables indicate number of labelled nuclei seen under microscopy: a) Results of experiment in December 1999 (epidermal renewal period); b) Results of experiment in June 2000 (epidermal non-proliferative period)

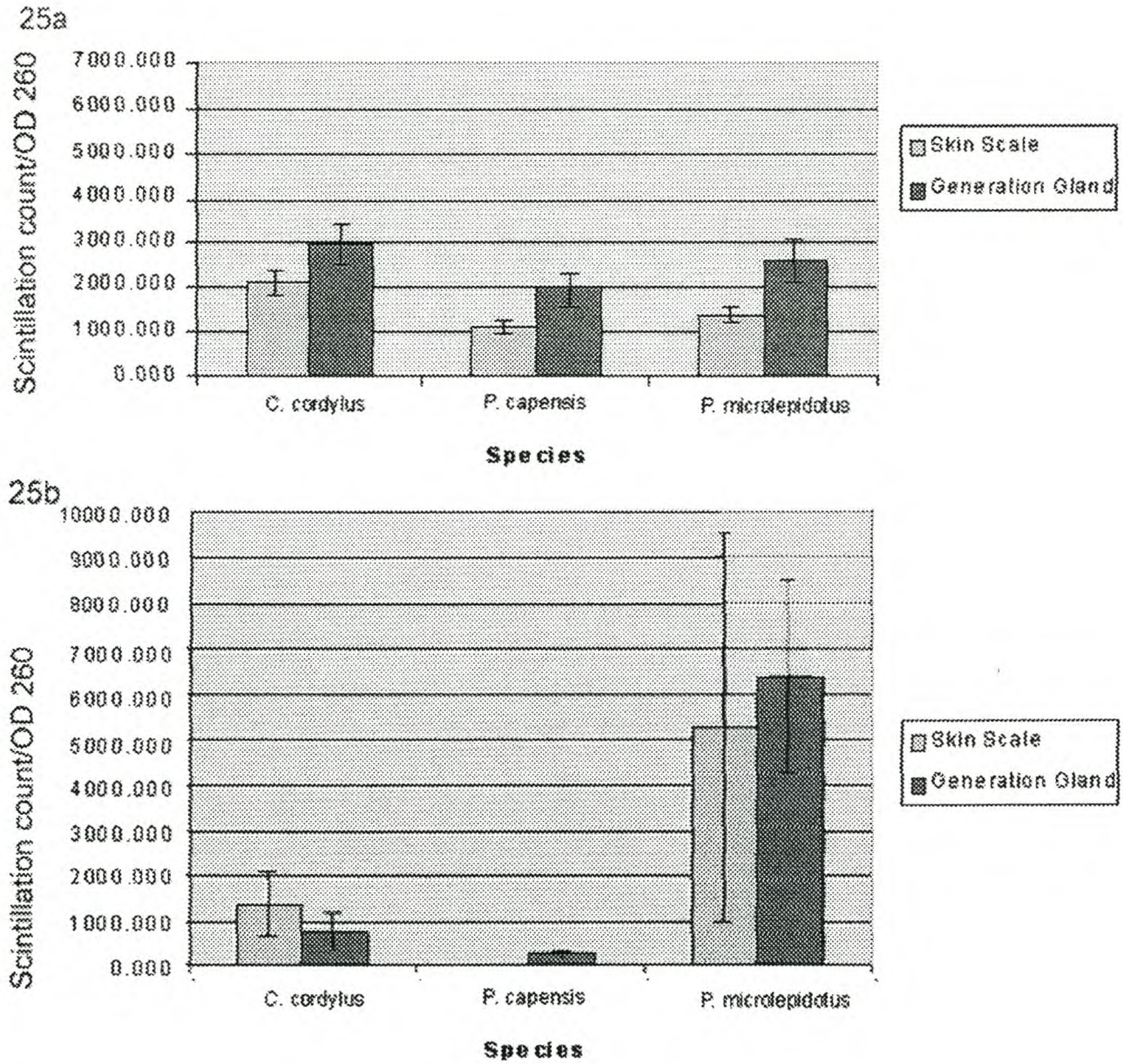


Figure 25. Results of labelled thymidine incorporation by the skin and generation glands of three cordylid species. Tables indicate incorporation of thymidine into tissue (scintillation count), expressed as ratio against available DNA (OD260) to counter effects of size: a) Results of experiment during theoretical proliferation period (December 1999); b) Results of experiment during theoretical non-proliferative period. (June 2000)

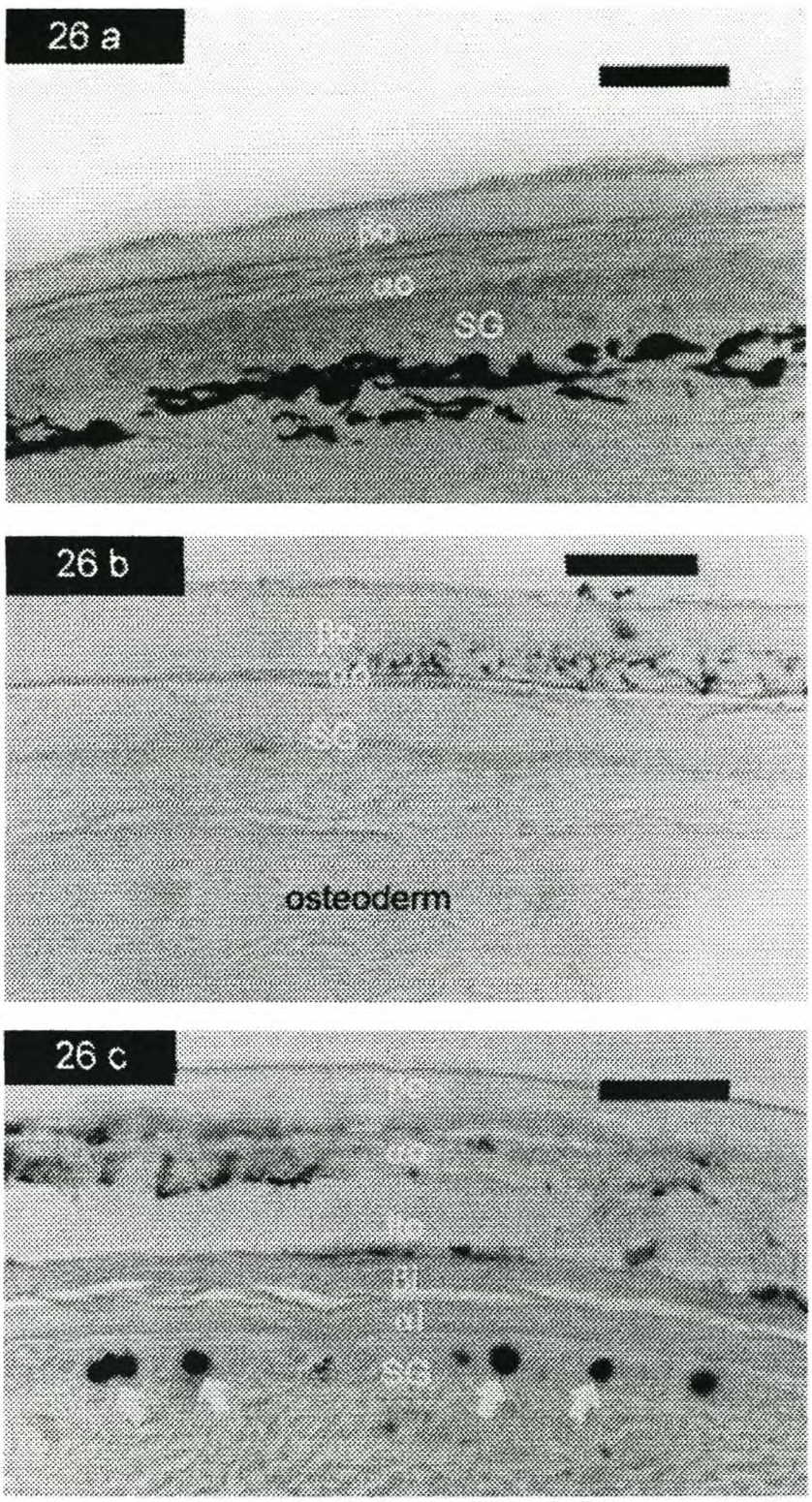


Figure 26, Unspecialized skin, illustrating positions of labelled nuclei in autoradiography experiment in December 1999: a) of *P. capensis*, displaying no radioactive labelling; b) *C. cordylus*, displaying no radioactive labelling; c) of *P. microlepidouts*, displaying radioactive labelling. Radioactively labelled nuclei indicated by yellow arrows. scale bar = 1000 μm.

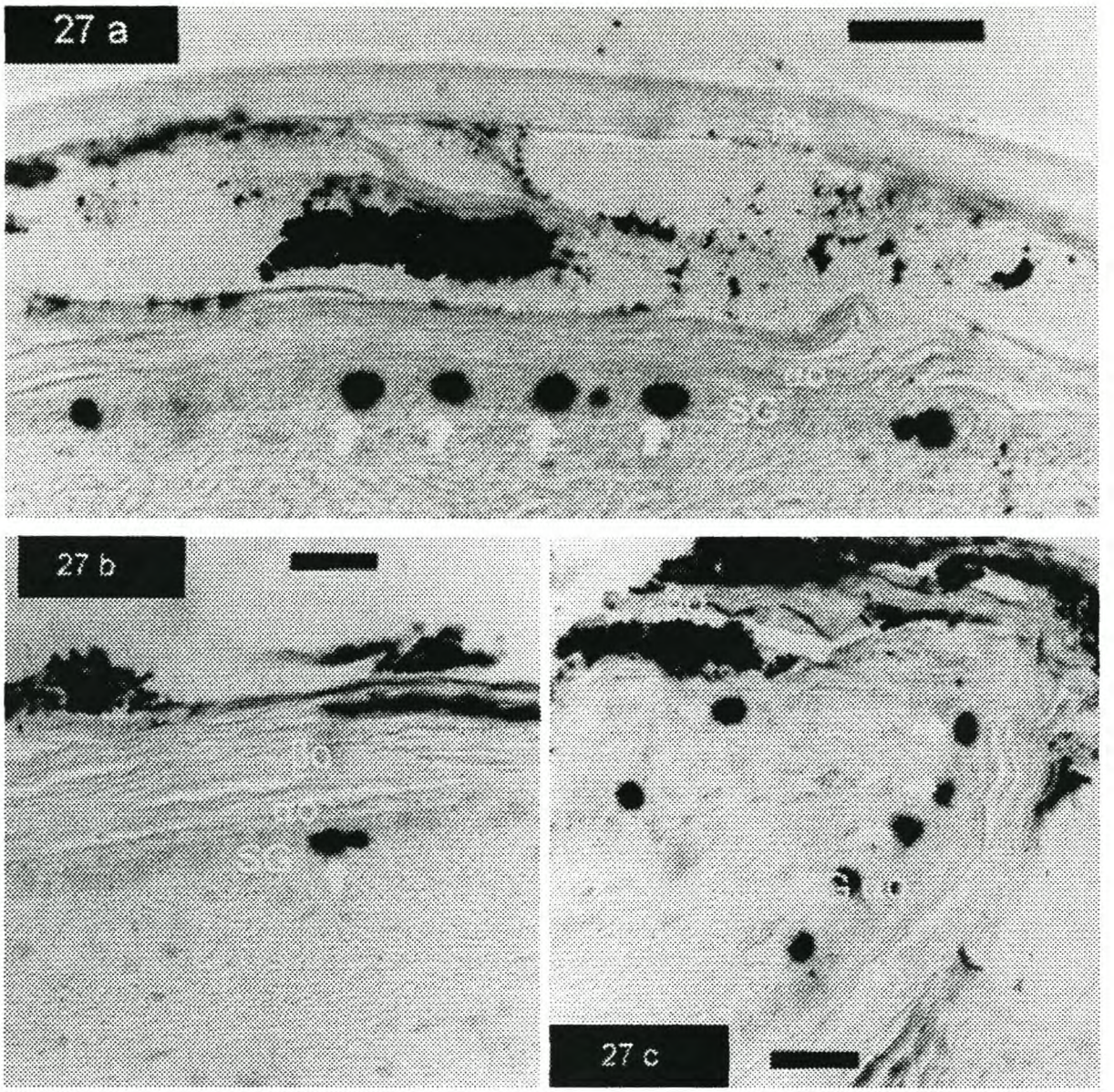


Figure 27. Unspecialized *P. microlepidotus* skin, showing PRS, illustrating positions of labelled nuclei in autoradiography experiment in June 2000: a) skin scale, showing PRS and high levels of radioactive labelling; b) outer scale surface, showing low levels of radioactive labelling; c) hinge region of the scale, displaying high levels of radioactive labelling. scale bar = 1000 μm.

FIVE

SUMMARY

Cordylids show little deviation from the generalized skin structure or skin shedding cycle. In comparison to the generalized skin structure of squamates, a mesos layer is not visible under light microscopy in cordylids. As the mesos layer was reported to be only 3µ thick in other lizards (Maderson, 1970), the lack of the mesos layer in cordylids is not considered to be certain, as light microscopy is possibly too 'coarse' a tool to determine the absence of such a layer. Cordylidae show no cytological changes from those seen in the five stages of the epidermal renewal phase described for gekkonids. There may be temporal differences in frequency of shedding and duration of renewal periods between gekkonids and cordylids, as the duration of the shedding cycle in cordylids, known to last 14 days in gekkonids, could not be determined using museum specimens in Cordylidae.

Epidermal glands in cordylids: histological investigation confirmed gross morphological studies (van Wyk & Mouton, 1992), showing that the layer producing the glandular material was the β-layer, and the presence of three types of generation glands. This indicates similarity between cordylids and gekkonids, in that both cordylid generation glands and gekkonid beta-glands produce glandular material via a differentiated β-layer (Maderson, 1968a, 1970). The other layers of the epidermal generation were also indicated to be present in the generation glands, an aspect unmentioned in van Wyk & Mouton (1992).

There is indication that growth progresses downwards during the pit glands' existence (*P. microlepidotus*). Dermal folds split the pit into several pits in some glandular scales. The pit appears to be continuously increasing in either depth or shape. Glandular material is only produced on the base of the pit, indicating that the growth of dermal folds cannot be progressing from the SG level upward, as the distal glandular layers that are complete cannot be formed from an SG already split into two smaller glandular producing areas by a dermal fold. What would appear to be the case is that the dermal folds grow up with generations surrounding them.

These different generation gland types correspond to different shedding cycles within the family, or different shedding frequencies. Anecdotal information (Mouton, pers. comm.; van Wyk, pers. comm.) was that cordylids shed once a year. Museum specimens, collected from each month of the year, were sampled to determine any information about shedding cycles of the three representative species used in this work. Shedding frequency patterns were shown in that cordylids display one period in a year when no shedding activity was observed in the unspecialized skin, followed by one period of epidermal renewal. The length of the dormant period (PRS) varies considerably between species (two to six months). It has been shown previously in gekkonids that stimulation for shedding may have to do with ambient temperature to some degree (Chiu & Maderson, 1980). There, warmer temperatures caused a reduction in the rest phase, increasing shedding frequency. In cordylids, the epidermal renewal period is in the warmer months while the skin appears dormant in winter.

Seasonal variation of the different generation gland types and their unspecialized skin scales was shown using autoradiographic techniques. When both *C. cordylus* and *P. capensis*' generation glands from the samples removed in December were compared with those removed in June, statistically significant differences were noted in both the relative mitotic activity and the radioactive labelled nuclei.

Generation glands appear to be linked to testosterone levels. This is seen by the activity levels of different generation gland types appearing to be affected by the circulating titer of androgens that vary with the testicular cycle. Androgen levels could also affect the shedding cycle of the skin (Maderson, pers. comm.). *P. microlepidotus* has a small peak in testosterone level in spring, and a large peak in autumn. The situation is reversed for *C. cordylus* and *P. capensis*, with a large peak in spring and a small peak in autumn (van Wyk, pers. comm.).

Pseudocordylus microlepidotus showed higher generation gland activity (radioactive labelling) in December, after the high peak in testosterone levels. *Pseudocordylus capensis* and *C. cordylus* showed significantly less labelling in this period, having just come off the low peak in testosterone levels. All species showed higher labelling in the December sample than in the June sample, but looking at the comparison between species, rather than intraspecific variation between seasons,

we can see that in June, *P. microlepidotus* showed much lower labelling than either *C. cordylus* or *P. capensis*. Here, *P. microlepidotus* had just come off the low peak in testosterone levels while the other two species had come off the high peaks in testosterone levels. Thus, it is clearly indicated that testosterone appears to affect generation gland activity.

Generation glands were shown to be asynchronous, with regard to the epidermal shedding cycle; with surrounding unspecialized skin scales. This asynchrony was shown on a histological level in chapters two and three, and from mitotic activity levels in chapter four. Asynchrony is described as: a generation gland in one stage of epidermal renewal while the surrounding unspecialized skin is in another. This was shown to be the case in all but six lizards of 143 specimens sampled. No other histological paper has shown that asynchrony exists between differentiated glandular scales and normal, unspecialized skin (although van Wyk & Mouton (1992) suggested it may be so), so Cordylidae appears to be the only family investigated to have asynchronous glandular scales.

FUTURE DIRECTIONS

Using end of PRS as a starting point, accurate determination of the duration of the shedding cycle of unspecialized skin scales is possible. Continuous biopsies from individual animals over the renewal period will also reveal how many times cordylids actually shed in an annual cycle. Preliminary investigation could be done by applying a stain, paint or dye to the epidermis, and seeing how many times it disappears during one renewal period. Paint should be applied to the body, not the head, as the head receives rougher treatment by the animal itself, and paints could be scratched off.

Tissue cultures can be performed with squamate skin (Flaxman et al, 1968) and skin biopsies from cordylids could be cultured and sampled at regular intervals to determine shedding stages. Addition of certain hormones to culture media can stimulate shedding to begin, and can possibly indicate whether the renewal phase in cordylids does in fact take 14 days as seen in gekkonids (Maderson, 1985).

Correlating generation gland activity (autoradiography) with circulating titer of androgens (Chiu et al, 1970; 1975) may reveal why cordylid generation glands show variation in activity levels, as shown in chapter 4.

The importance of asynchrony cannot be underestimated, as this family is the only family thus far to have this feature described. This feature provides investigative opportunity into control of individual scale types and specific areas of the epidermis, and possible evolutionary information. Generation glands are now known to be asynchronous with the normal unspecialized skin, but longer serial sections are needed to investigate the state of synchrony within a gland patch on an individual single lizard. Serial sections through the generation gland in this thesis were made through only one gland to describe the structure of generation glands. At the beginning and end of each series, some sections were made through adjacent glandular scales, and these appeared to be asynchronous from the gland sectioned, but too few sections were made of adjacent glands to be positive about this feature.

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