APPLICATIONS OF MICROMORPHOLOGY TO NATRICINE SYSTEMATICS (SERPENTES, COLUBRIDAE): VISUAL CELLS AND SCALES

By

RANDALL HOWARD STOVALL

Bachelor of Science
University of Texas at Arlington
Arlington, Texas
1973

Master of Arts
University of Texas at Arlington
Arlington, Texas
1975

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment for
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July, 1982

APPLICATIONS OF MICROMORPHOLOGY TO NATRICINE SYSTEMATICS (SERPENTES, COLUBRIDAE): VISUAL CELLS AND SCALES

Thesis Approved:

Rudolph J. Miller
Thesis Adviser

Larry D. Talbot

Bryan P. Glass

Stanley Fox

Norman N. Durham
Dean of the Graduate College

PREFACE

Microscopic anatomy of natricine snake retinae and scales was interpreted in terms of systematic and ecological relationships. Both light and electron microscopy of visual cells and retinae were utilized. Scales were examined by scanning electron microscopy. Species were selected to provide a representative survey of the North American Natricinae (watersnakes and related species) as well as certain ecologically similar species such as the mudsnake (Farancia abacura) and cottonmouth (Agkistrodon piscivorus). Visual cell patterns were determined to be essentially as predicted by other workers and not particularly useful systematically within the group. Scale surface morphologies presented here provide the largest survey of snake scale surfaces yet published. Scale surface patterns are complex and show interspecific and intergeneric variations.

Many people have contributed to the completion of this project. The most important and significant supporters have been my parents, Mr. and Mrs. Jack W. Stovall, and my wife, Laraine. These three have helped in many ways and have litterally kept me going. I thank them.

Dr. J. P. Kennedy gave freely of his advice and time during my work in Houston, Texas (1975-1978). He also helped with collection of specimens and assisted with

experiments. To him I extend a sincere thank you.

I thank Dean Hector, Jim Lish, and Mike Mullins for spending their time to discuss various aspects of the study and for offering useful suggestions.

The staff at the OSU Veterinary College was most helpful in providing facilities for scanning electron microscopy.

I would like to thank my committee members in the School of Biological Sciences. I have enjoyed and profitted from my interactions with them all. Dr. Milton Curd served on my committee from 1978 until his death in 1981. Dr. Curd was an excellent zoologist, teacher, and friend whose passing is mourned by all who knew him. Dr. Larry Talent graciously agreed to replace Dr. Curd on the committee. Dr. Bryan Glass was both an inspiring teacher and a good supervisor during my teaching of histology labs. Dr. Rudolph Miller was most stimulating in discussions of evolutionary biology in and out of the classroom. My dissertation supervisor, Dr. Stanley Fox, has always gone out of his way to seek answers and solutions to my problems while requiring critical thought and meaningful work on my part.

Financial support for my research was provided by the University of Texas Health Science Center at Houston (1975-1977) and the School of Biological Sciences, Oklahoma State University (1978-1979).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Visual cells and retinae of six species of watersnakes and related species were studied (Nerodia erythrogaster, Nerodia fasciata, Nerodia rhombifera, Regina grahami, Storeria dekayi, Thamnophis proximus) along with four ecologically related species (Agkistrodon contortrix, Agkistrodon piscivorus, Farancia abacura, Pelamis platurus). Eyes were studied with both light and transmission electron microscopy (TEM). Primary data were taken from the light microscope observations with TEM data used to provide a general description of the ultrastructure of natricine visual cells.

Scanning electron microscopy was utilized to study the surface morphology of dorsal mid-body scales of 21 species of natricine snakes and 3 ecologically related species (Table I and Appendix A). Data from retinal and scale study were interpreted in terms of the following questions. Are retinal and scale surface patterns similar among natricine snakes? Do snakes with similar habits have similar retinal and/or scale surface patterns? At what level (if any) are retinal and scale patterns useful systematically? Do semi-aquatic snakes have a distinctive retinal and/or scale

TABLE I

STUDIES OF SNAKE SCALE SURFACES (PUBLISHED ELECTRONMICROGRAPHS)

Taxon (number of species studied)	Authority
BOIDAE	
BOINAE	
Boa (2)	Hoge and Santos (1951)
Epicrates (1)	Hoge and Santos (1951)
Eunectes (1)	Hoge and Santos (1951)
COLUBRIDAE	
COLUBRINAE	
Drymarchon (1)	Monroe and Monroe (1968)
NATRICINAE	
Nerodia (5)	Stovall (this study)
Regina (4)	Stovall (this study)
Storeria (2)	Stovall (this study)
Thamnophis (9)	Stovall (this study)
Tropidoclonion (1)	Stovall (this study)
XENODONTINAE	
Farancia (1)	Stovall (this study)
UROPELTIDAE	
Rhinophis (1)	Gans and Baic (1977)
VIPERIDAE	
CROTALINAE	
Agkistrodon (2)	Stovall (this study)
Crotalus (2)	Stewart and Daniel (1975)

surface pattern?

Representative literature on natricine systematics and ecology as well as snake eyes and scales is reviewed below.

Systematics

Historically modern snake taxonomy dates from the work of Boulenger and Cope in the late 1800's (for reviews see: Dowling and Savage, 1960; Rossman, 1963, 1967; Dowling, 1967; Underwood, 1967a). Water snakes and their allies are a distinct group of snakes. Cope (1895:200) defined the Natricinae as "colubrid snakes with hypapophyses on all dorsal vertebrae and with noncalyculate hemipenes that are minutely spinose but bear one or more enlarged basal hooks." According to Rossman (1963), subsequent to Cope, highly influential opinions were expressed by Dunn (1928), Bogert (1940), and Malnate (1960). Underwood (1967a) reviewed snake classification in general, utilizing over 20 different characters to develop a broader classification that elevated water snakes to family level (Natricinae). Most recent authors have retained a Copeian concept however (Rossman, 1963; Dowling, 1967, 1975; Rossman and Eberle, 1977; Dowling and Duellman, 1978).

Agreement has been lacking on the generic composition within the subfamily. Dowling (1975) included 9 genera of New World Natricinae, of which all except Adelophis is represented in North America (Table II). In addition to the genus list, Table II also includes important systematic ref-

TABLE II

NATRICINE GENERA OF THE NEARCTIC

Genus (number of species)	Systematic Reference
Adelophis (1)	Taylor, 1942
Clonophis (1)	Rossman, 1963a
Natrix (now called Nerodia) (8)	Rossman and Eberle, 1977
Regina (4)	Malnate, 1960; Rossman, 1963a
Seminatrix (1)	Dowling, 1950
Storeria (4)	Trapido, 1944
Thamnophis (22)	Denburg, 1918; Rossman, 1961, 1963b
Tropidoclonion (1)	Conant, 1975
Virginia (2)	Blanchard, 1923

Source: H. G. Dowling, 1975. A provisional classification of snakes. Yearbook of Herpetology 1:167-170.

erences for each genus. To my knowledge no family tree or dendrogram of phylogenetic relationships has been constructed for the group as a whole.

The composition and definition of the genus Natrix has been especially difficult. Rossman and Eberle (1977) have presented the most recent review of Natrix and suggested extensive changes in the genus. Prior to 1960 the genus Natrix comprised a group of 86 species worldwide. Malnate (1960) divided the heterogeneous genus on the basis of differences in the sulcus spermaticus (a hemepenial character; see Dowling and Savage, 1960), maxillary dentition, and internasal size into five genera (Amphesma, Fowlea, Macrophis, Natrix, and Rhabdophis). All except Natrix were confined to Old World distribution. Natrix still retained 21 species distributed through the Old World and North America.

In the same year Smith and Huheey (1960) proposed to further reduce the genus Natrix by resurrecting the genus Regina and placing in it four of the North American species (grahami, kirtlandi, rigida, septemvittata). Citing differences in color pattern, form and proportions (including head width, eye size, pupil size, head length/body length ratio), scutellation, hemipenes, osteology, and dentition, Rossman (1963a) supported the separation of Regina from Natrix and redefined the genus to include alleni and removed kirtlandi for placement in the monotypic genus Clonophis.

The generic status of Regina was further supported

by biochemical analysis of blood hemoglobin (Sutton, 1969) and study of cranial musculature (Varkey, 1973). A perusal of recent literature shows the genus to be generally accepted. Not all systematists, though, seem to be convinced by the arguments of Smith and Huheey and Rossman; Conant (1975) failed to recognize the genus in his latest edition of A Field Guide to Reptiles and Amphibians of Eastern and Central North America.

Biochemical techniques (microcomplement fixation and immunoelectrophoresis) were employed to study blood proteins in a number of snakes (George and Dessauer, 1970;Mao and Dessauer, 1971; Minton and Salanitro, 1972; Minton, 1976). Mao and Dessauer (1971) argued that the magnitude of blood protein differences among species correlates with their length of reproductive isolation. Blood protein differences thus indicate a pronounced separation between Old World and New World species of Natrix. New World Natrix are more closely correlated to the New World natricine genus Thamnophis than to any Old World Natrix. This agrees with the view that New World Natrix are ancestral to Thamnophis (Malnate, 1960; Mao and Dessauer, 1971).

Karyological data (Hardy, 1971; Baker, Mengden, and Bull, 1972; Eberle, 1972; Rossman and Eberle, 1977) also show a separation between Old World and New World Natrix and a close correlation among all New World Natricinae. Variation in chromosome number indicates three separate groups of Natrix; an Asian group (2n = 42), a European

group (2n = 34), and a North American group (2n = 36, the same as all New World natricine species for which data are available).

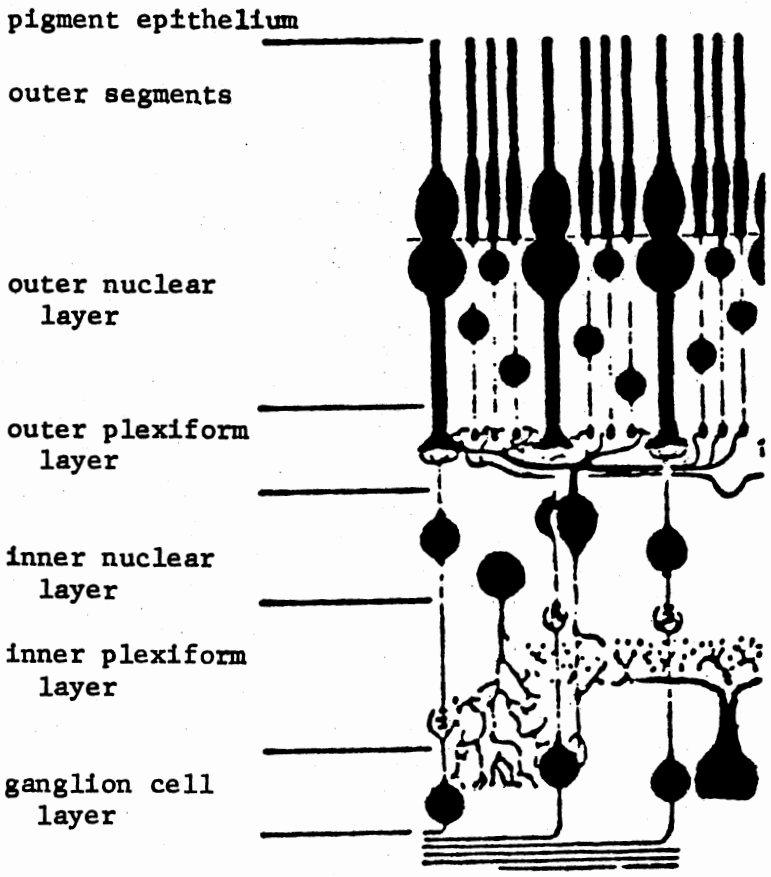
Rossman and Eberle (1977) reviewed morphological data as well as biochemical and karyological data related to Natrix. They concluded that the genus should be divided into four distinct genera (Afronatrix, Sinonatrix, Natrix, Nerodia). The 8 North American species were placed in Nerodia and included along with other New World Natricinae in the tribe Thamnophiini.

In addition to the morphological, karyological, and biochemical comparisons above, other characters have been used in the study of squamate systematics. Underwood (1967a, others) used pancreas, kidneys, carotid arteries, and visual cell types in his analyses. Biochemical analysis of venom has been used in studies of venomous species (e.g. Foote and MacMahon, 1977) as well as analysis of scent gland secretion lipids (Oldak, 1976). Ultramorphology of scale surfaces has been used in lizard studies (Burstein, Larsen, and Smith, 1974; Cole and VanDevender, 1976) and to a very limited extent in snakes (Hoge and Santos, 1951).

Visual Cells

A diagram of a generalized vertebrate retina, based on Walls' (1942) work, is included for reference (Figure 1). A more recent summary of the literature on the vertebrate

Figure 1. Generalized Diagram of Vertebrate Retina (from Walls, 1942)


pigment epithelium
outer segments
outer nuclear layer
outer plexiform layer
inner nuclear layer
inner plexiform layer
ganglion cell layer

retina may be found in Rodieck (1973). Vertebrate photoreceptor cells, rods and cones, are similar in many aspects and a description of a generalized squamate visual cell follows (Figure 2). The photopigment-containing outer segment is the most scleral element and is in contact with the pigment epithelium (the outermost or most scleral layer of the retina). The dimensions of the outer segment differ substantially in rods and cones. Between the outer segment and the nucleus is the inner segment. An oil droplet is present in some reptiles in the scleral end of the inner segment. In snake visual cells, however, the scleral end of the inner segment is entirely filled by the heavily staining ellipsoid, a mitochondrial body. An ovoid, glycogen-containing body is present in some lizards in the inner segment adjacent to the vitread region of the ellipsoid, but this paraboloid has not been described in any snake. An amorphous region, the myoid, is located between the ellipsoid and the nucleus. This region is capable of extension in those vertebrates with retino-mechanical light and dark adaptation. Snakes, however, lack myoid extensibility. The synaptic region of the receptor cell is located vitread to the nucleus.

Rods are visual cells which mediate low illumination vision and generally have a larger outer segment which may serve to increase sensitivity to light. The synapses of rods tend to be highly convergent; i.e., several rods synapse with a single bipolar cell. This convergence

Figure 2. Generalized Diagram of Squamate Visual Cells

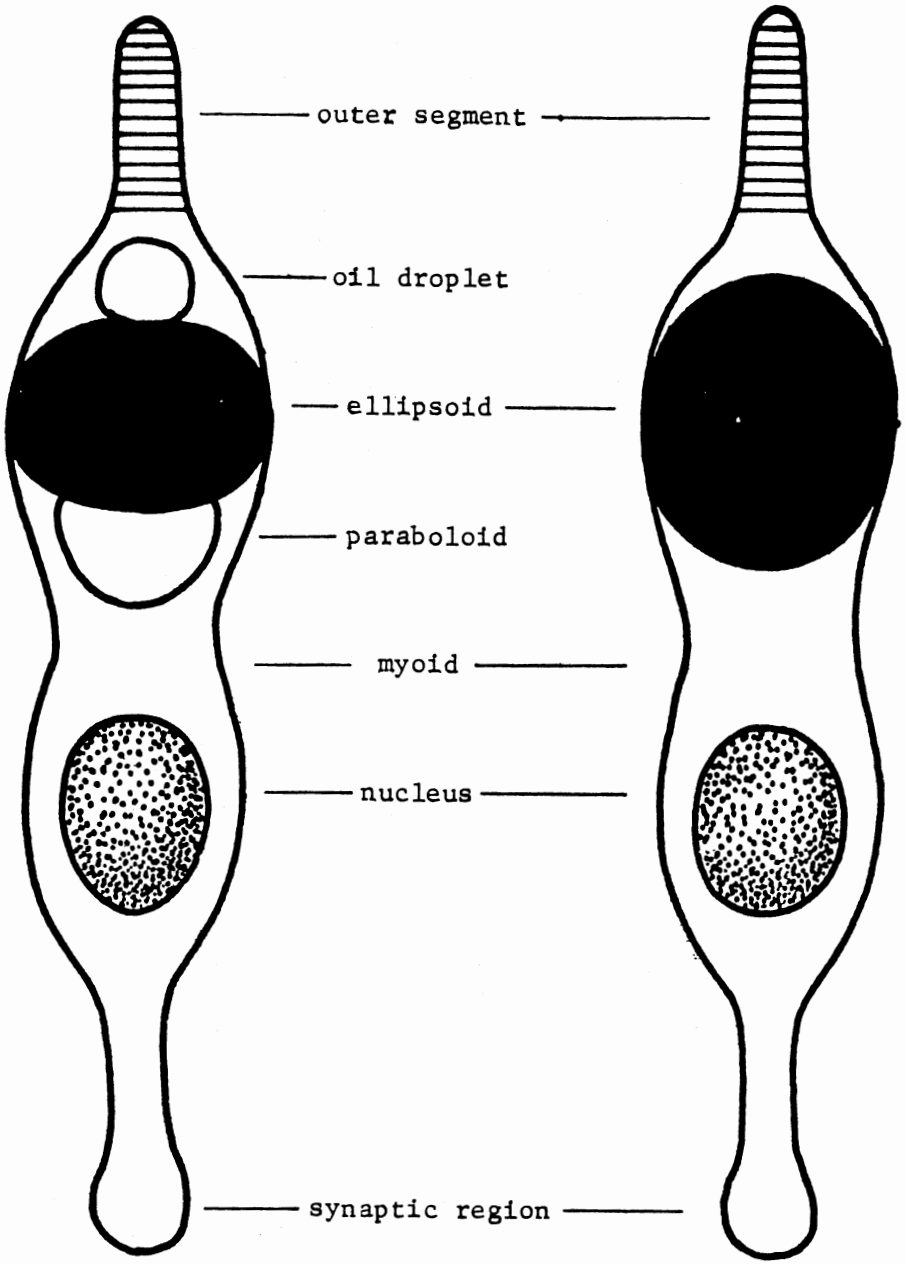
a.) Diurnal Lizard

b.) Snake

In both a. and b. the part of the visual cell generally called the "inner segment" includes the region from nucleus to outer segment.

a.)

b.)


outer segment
oil droplet
ellipsoid
paraboloid
myoid
nucleus
synaptic region

increases the sensitivity of rod-mediated vision (Walls, 1942).

Cones are visual cells mediating bright light vision. Their outer segments are smaller than those of rods, possibly resulting in a higher stimulation threshold and decreased sensitivity. Cone synapses tend not to be convergent; each cone contributes more to the sensory output of the retina than does each rod. This suggests that cone-mediated vision is more acute than rod-mediated vision (Walls, 1942).

The eyes of snakes have been studied by relatively few authors. Walls (1942) was possibly the most prodigious student of vertebrate eyes and his monograph, The Vertebrate Eye and Its Adaptive Radiation, is a classic. Walls reported observations on over 100 species of reptiles, including 21 species of snakes. Walls' observations were limited to light microscopy because electron microscopical techniques were not available during the period of his research. Nevertheless, Walls advanced useful theories explaining the structure of eyes and visual cells of snakes, as well as intragroup variations.

Underwood (1960, 1966, 1967a,b, 1970) enlarged on Walls' work, and included observations on 38 additional species of snakes (Appendix B). Underwood utilized both light microscopy and, to a limited extent, electron microscopy. Based on his (and Walls') light microscope observations, Underwood (1967a,b) constructed a phylogeny of

snake visual cells and further stated that visual cells and visual cell patterns offer useful taxonomic characters for snakes. A review of the literature, however, indicates that the visual cells of comparatively few species of snakes have been studied (Appendix B).

Walls' (1942) description of snake visual cells has served as the standard reference for all subsequent studies; a review of his designations of visual cell types is useful. Colubrids generally have a "diurnal" type retina, which contains cone cells of three types (no rods). These were designated by Walls as Type A, Type B, and Type C (Figure 3). Type A cones are large single cones; i.e., typical snake cones. These possess somewhat bulbous inner segments, which in stained sections are almost completely darkened by the densely staining ellipsoid. The outer segment is short and conical. Type B cones are actually two closely associated cells, or "double cells". The two cells are unequal in size as well as substructure. The larger cell is the chief cell; the small cell is the accessory cell. Chief cells are essentially identical morphologically to Type A single cones. Accessory cells are very slender, have a reduced ellipsoid and possess a unique paranuclear body between the nucleus and the ellipsoid. As Walls noted, the paranuclear body stains densely, like a normal ellipsoid, and later studies have shown a similar substructure (Stovall, 1975, 1976a). The presence of the paranuclear body is useful in diagnosing visual types since it indicates

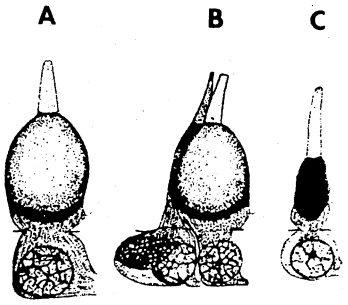
Figure 3. Snake Visual Cell Types
(from Walls, 1942)

a.) Type A Single Cone

b.) Type B Double Cones

c.) Type C Single Cone

Notice that Type B cones consist of two closely associated visual cells: a smaller accessory cell and a larger chief cell.


A
B
C

the presence of Type B cones. The third cell type, Type C, is a smaller single cone. Type C cones are more slender, often shorter, and usually less abundant than Type A cones.

Some snakes, especially non-colubrids, possess additional types of visual cells. Crotalids possess visual cells that suit them better for nocturnal activity. Crotalid retinae possess Type A and Type B cones which are more elongate and slender than those of colubrids. Furthermore, the Type C cells are not cone-like, instead they are elongate with an inner segment (ellipsoid region) the same diameter as the outer segment. The Type C outer segments are long and do not taper. Walls called these Type C rods.

Viperids differ from the standard colubrid retina even more. A typical viperid retina contains Type A, B, and C cones and a fourth cell type Walls called Type C'. The C' cell is quite similar to the Type C rod of crotalid; accordingly Walls called them Type C' rods. (Underwood [1970] called this a Type D cell.)

Scales

That snakes are covered by epidermal scales is fundamental. The arrangement and number of scales have been widely utilized in systematics. The structure of scales and squamate epidermis has been described at both light and electron microscopic levels (Maderson, 1964, 1965a,b, 1966; Roth and Jones, 1967, 1970; Maderson, Flasman, Roth, and Szabo, 1972). Maderson's (1964, 1965a) reviews of squamate

epidermis are used for the following description (Figure 4). Just after shedding has occurred the epidermis consists of two parts, an outer epidermal generation and a basal stratum germinativum. The outer epidermal generation consists of two layers of keratin and two layers of living cells.

The keratin layers, β and α (outer and inner layers, respectively) are composed of dead, keratinized cells and are named for the type of keratin they contain (Rudall, 1947). The β-keratin is very hard and inflexible and is the major layer on outer scale surfaces, whereas the α-keratin is more flexible and predominates on inner scale surfaces and inter-scale regions of the epidermis. In histological sections β-keratin does not stain in hematoxylin and eosin, but α-keratin stains pink. The outermost layer of dead keratinized cells in the β-layer is the oberhäutchen, a layer characterized by various ridges and folds which appear as serrations in transverse sections.

The lower portion of the α-layer and the living cells of the outer epidermal generation collectively compose a stratum intermedium which, at this stage, i.e., immediately after shedding, is bordered by the stratum germinativum medially. This resting stage in the ecdysis cycle, as just described, lasts some 3/4 of the total time between successive sloughs. At the end of the resting stage, the stratum germinativum undergoes rapid cell division producing a new layer or inner generation of the epidermal cells between itself and the stratum intermedium. The outermost layers of

Figure 4. Squamate Epidermis
(from Bellairs, 1969)

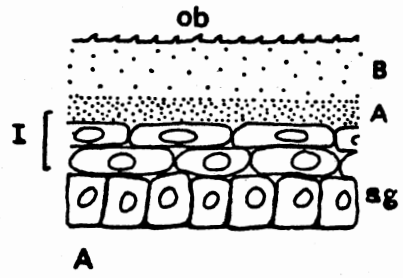
a.) Resting Stage

b.) Initial Development

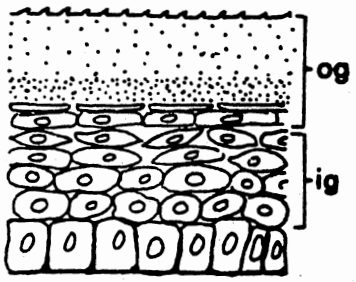
c.) Stratum Intermedium Degeneration

d.) Slough and New Outer Generation

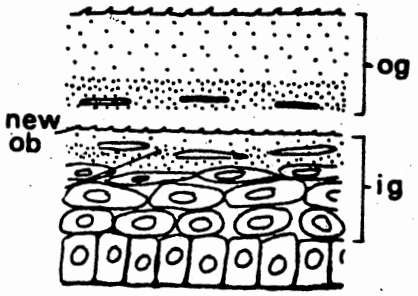
A = α-keratin layer
B = β-keratin layer
I = stratum intermedium
ig = inner epidermal generation
ob = oberhautchen
og = outer epidermal generation
sg = stratum germinativum



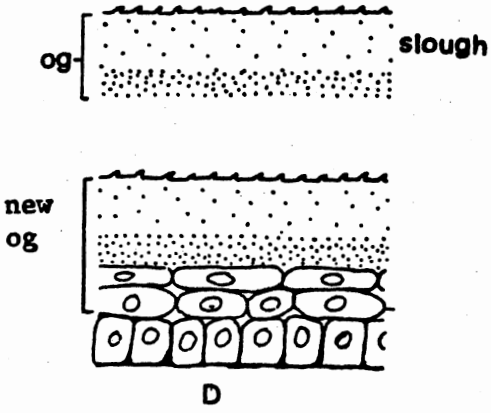
A



B



C



D

this new, inner epidermal generation keratinize and become new oberhautchen, β, and α layers. Shedding occurs when the stratum intermedium degenerates and the old outer epidermal generation is no longer attached to the underlying skin. After shedding the old inner epidermal generation becomes the new outer epidermal generation.

The oberhautchen of squamate scales has received attention because of the distinctive pattern of micro-ornamentation (folds and ridges). Hoge and Santos (1951) made parlodion replicas of the outer surfaces of the scales of 10 species of boid snakes and observed these replicas with a transmission electron microscope, although micrographs of only 4 species were published. They suggested that the oberhautchen micro-ornamentation occurs in species-specific patterns. Since the work of Hoge and Santos, few studies of micro-ornamentation of squamate scales have been reported. The availability of scanning electron microscopy (SEM) in recent years has made high resolution observations of scale surfaces relatively simple. In 1974, Burstein, Larsen and Smith published an account of their SEM study of scale surface morphology on 51 species of Sceloporus (Iguanidae). They too supported the value of oberhautchen surfaces in squamate systematics.

To minimize variation, Burstein et al. observed only those scales taken from the dorsal midbody region of the specimens, because micro-ornamentation probably varies on scales of different body regions of a given individual

(Stewart and Daniel, 1973). Burstein et al. utilized museum specimens and they showed the value of future studies utilizing the relatively unlimited potential of museum collections. Stewart and Daniel (1973, 1975) studied scale surfaces on several lizard species and found surface patterns (micro-ornamentation) to generally conform to current taxonomic arrangements for lizards.

A major problem not recognized by Burstein et al. (1974) is the effect of mechanical abrasion and erosion on oberhautchen surfaces during various periods in the ecdysis cycle. Cole and VanDevender (1976) studied Sceloporus scales with a scanning electron microscope and found that many of the "distinctive" features of oberhautchen surfaces reported by Burstein et al. were probably variations produced by abrasion. Thus the most easily compared surface patterns would be those of scales newly exposed immediately after shedding. Nevertheless, species discrimination was still possible, even for Burstein et al.

Oberhautchen surfaces have been implicated in various adaptive functions. Gans and Baic (1977) stressed the importance of scale surface morphology in locomoter adaptations. Soule and Kerfoot (1972) suggested that the micro-ornamentation of oberhautchen increases the surface area of squamates. They went on to argue that larger scales may have more elaborate micro-ornamentation and hence even greater surface area than one would expect on the basis of size alone. They related scale size and thus surface area

of oberhautchen to thermal adaptations. Horton (1973) challenged this interpretation, however, by presenting seemingly conflicting data. Also, scales have been shown to be effective filters of ultraviolet radiation (Tercafs, 1963).

Ecology

The subfamily Natricinae is named for the semi-aquatic genus Natrix, whose members are commonly called watersnakes. All members of the subfamily, however, do not possess an equal affinity for water. The species included here show a range of habitat and food preferences.

Nerodia is widely distributed in central and eastern North America and most watersnakes spend much of their time in or near water (see Conant, 1975, for general reference). Watersnakes typically bask on logs or brush near the water to which they escape when alarmed. Species of Nerodia are confined to freshwater, except N. fasciata subspecies, which occupy coastal areas bordering the Gulf of Mexico. Nerodia cyclopion, fasciata, rhombifera, and sipedon are primarily piscivorous, whereas N. erythrogaster preys more heavily on frogs (Diener, 1957; Brown, 1958; Preston, 1970; Mushinsky and Hebrard, 1977a; Kofron, 1978; Mushinsky, Hebrard, and Wally, 1980). Most watersnakes are quite aggressive when encountered in the field.

Regina has a similar distribution to Nerodia in North America. Species of Regina differ from Nerodia in both

behavior and diet. Regina are secretive and generally not aggressive when encountered (see Conant, 1975). Crayfish are the primary (some authors say exclusive) food utilized by all four species (Strecker, 1926; Rossman, 1963; Hall, 1969; Branson and Baker, 1974; Conant, 1975; Franz, 1977; Mushinsky and Hebrard, 1977a; Kofron, 1978; Godley, 1980). Regina species show a range of habitat and behavioral preferences, however. Regina alleni and rigida are very secretive and often inhabit dense vegetation such as water hyacinths or sphagnum mats. Both are considered somewhat fossorial as well. Regina grahami and septemvittata are less secretive, less fossorial, and are more frequently encountered, especially in debris near water's edge or in crayfish chimneys (Strecker, 1926; Rossman, 1963; Conant, 1975).

Storeria is distributed throughout the eastern half of North America from the Great Lakes to the Gulf Coast and into Mexico. The common S. dekayi is found in a variety of habitats from river-bottoms to uplands (Conant, 1975). This snake is also encountered in city lots, parks, etc. (Trapido, 1944; Conant, 1975). Storeria occipitomaculata is more secretive and of spotty distribution. Diet of both species includes earthworms and soft-bodied invertebrates (Conant, 1975).

Thamnophis is the largest genus of Natricinae and accordingly its ecology diverse. The genus is represented throughout North America from wetlands to uplands. Further,

garter snakes range from semi-aquatic to terrestrial in habits. Thamnophis couchi and T. elegans are so similar morphologically that they have been considered a single species, although in habits couchi is distinctly aquatic and elegans is terrestrial in preference (Rossman, 1963a; Shaw and Campbell, 1974). General accounts of the genus may be found in Denburgh (1919), Ditmars (1931), Carpenter (1952), Conant (1975), and others.

Garter snakes (genus Thamnophis) are often semi-aquatic and in the western, more arid portions of their range often commonly called "watersnakes". In the more mesic eastern part of the continent, however, they are found in all types of habitats. Diet generally consists of invertebrates, such as earthworms or leeches, amphibians, fish, or even small mammals or birds (Conant, 1975).

Tropidoclonion lineatum is closely related to Thamnophis in habits, diet, and morphology (Shaw and Campbell, 1974; Conant, 1975). The lined snake ranges through central North America. This secretive snake is generally found under rocks, brush, and other debris and may occur in city lots as well as rural areas. Diet includes earthworms and soft-bodied insects (Strecker, 1926).

Snakes of three non-natricine groups were also included in the study: Agkistrodon, Farancia, and Pelamis. Agkistrodon is a genus of crotalid snakes distributed through southern North America from Texas to Florida. A. contortrix, the copperhead, is more terrestrial, being found well

away from water, except in the driest portions of its range (Dowling, 1975). A. piscivorus, the cottonmouth, is quite similar to Nerodia species in habitat preference. Cottonmouths are semi-aquatic, always found in or near water where they may bask on branches, logs, or stones at water's edge. Their diet is more generalized than most watersnakes, however (fish, frogs, snakes, lizards, turtles, small alligators, small mammals, small birds [Conant, 1975]).

Farancia abacura, the mud snake, is a colubrid in the subfamily Xenodontinae. Mudsnakes are distributed through southeastern North America from east Texas to Virginia. Farancia abacura is a secretive, fossorial, swamp snake whose diet includes sirens, salamanders, fish, and frogs (Strecker, 1926; Conant, 1975). The tip of the tail ends in a sharp point which is thought to be used in subduing prey, but is also used on collectors (Conant, 1975). Further, F. abacura is unique among snakes in attending the nest after eggs are laid. Apparently the female digs a burrow, lays the eggs, and then remains in or near the burrow until the eggs hatch (Riemer, 1957).

Finally, Pelamis platurus, the yellow-bellied sea snake, is an elapid sea snake in the Hydrophiinae (Dowling, 1975). Pelamis is the "most pelagic of the extant reptiles, being born alive at sea and perhaps never going ashore during its lifetime" (Hibbard and Lavergne, 1972). Diet consists chiefly of fish (Klauber, 1935).

Types of Ecological Comparison

The species studied allow comparison of both semi-aquatic and terrestrial natricine snakes. This facilitates analysis of the possible influence of aquatic or terrestrial habits on visual cell types and scale surfaces. To enhance such analysis, aquatic, non-natricine species were also studied. The species studied may be grouped into three broad groups on the basis of aquatic, semi-aquatic, or terrestrial habits: (1) Pelamis, (2) Agkistrodon, Farancia, Nerodia, Regina, some Thamnophis, (3) Storeria, some Thamnophis, Tropidoclonion.

Snakes included in the study also show variation in proclivity for burrowing. Farancia and Regina alleni are especially fossorial (Strecker, 1926; Riemer, 1957, Rossman, 1963). Furthermore, Rossman (1963) stated that proclivity for burrowing varies in Regina with alleni and grahami representing the extremes (most and least fossorial, respectively).

Snakes may also be compared on the basis of general activity preference; i.e., diurnal, nocturnal, or secretive. Most natricine snakes are diurnal, although some Nerodia show a preference for night activity during hot weather. Pelamis is apparently diurnal from accounts of sailors and collectors. Agkistrodon piscivorus is primarily nocturnal but may be encountered in daytime. Farancia abacura, Regina alleni, R. rigida, and Storeria dekayi are secretive; all are rather infrequently encountered in the open.

Diet preferences (listed earlier) of the species studied are generally rather broad. Therefore, diet does not seem a fruitful area of comparison for the data presented here.

Adult specimens were studied without regard for size, age, or sex.

CHAPTER II

MATERIALS AND METHODS

Specimens

Snakes used in the retinal study were collected in the field by the author or associates (Appendix C). Snakes used in the scale study were preserved specimens from the collection in the Oklahoma State University Museum (Appendix A).

Laboratory Techniques

Tissue for retinal studies was removed from specimens recently killed by injection of lethal doses of sodium pentabarbital or decapitation following hypothermia. Fixation was usually accomplished by glutaraldehyde followed by osmium tetroxide. Except for eyes of Storeria dekayi and Agkistrodon piscivorus, which were embedded whole and serial sectioned, the eyes were diced during or after fixation and subsequently embedded in Spurr Low Viscosity Resin (Polysciences, Inc., Warrington, PA). Such specimens were then sectioned on an ultramicrotome and thick (ca. 1 μm) or thin (600 - 900 Å) sections were made for light and electron microscopy, respectively. Specimens for paraffin and/or celloidin embedment were fixed as above or in Kolmer's

fixative (Walls, 1938). Standard techniques were employed to process and embed the fixed tissue (Dawes, 1971; Humason, 1972). Sections were then cut on a rotary microtome.

Electron microscopic observations and micrographs were made on a Joel 100B transmission electron microscope (The University of Texas Health Science Center at Houston) or on a Zeiss 9S transmission electron microscope (The University of Texas at Arlington). Light micrographs were made using a Nikon automatic photometer system mounted on a Nikon light microscope.

Tissue for epidermal scale studies was obtained from preserved specimens stored in 50% isopropanol for varying lengths of time; all specimens were processed during summer, 1979. The scales were removed from the specimens and dehydrated to absolute ethanol and cleaned by sonication for 30 minutes as described by Cole and VanDevender (1976). Once cleaned the scales were dried between layers of KimWipe in a vacuum chamber overnight. Dry scales were coated with gold-paladium in a Hummer II sputterer and observed in a Jeol scanning electron microscope (College of Veterinary Medicine, Oklahoma State University). Scanning electron micrographs were made with Polaroid positive-negative film.

Light microscope counts and measurements were made using a filar micrometer for reference. Counts of receptor, interneuron, and ganglion cell nuclei were done on 400X fields. Ten fields were counted for each species. Analysis of photoreceptor types was made using oil-immersion (1000X)

fields in addition to the usual 400X fields.

Sections were stained with toluidine blue (epoxy) or Mallory's Triple stain and hematoxylin and eosin (paraffin and celloidin). Epoxy and paraffin or celloidin sections have different appearances due to differences in thickness (ca. 1 μm and 6 μm, respectively) and staining. Such differences affect the relative ease of cell type identification and the absolute number of cells present in a given section. Data used to compare different species were corrected for these numerical differences by the use of cell frequencies; i.e., total cell counts were not compared.

Precise orientation of sections on the retina was not possible due to processing methodology employed.

Phenetic Analysis

Data presented in the scanning electron micrographs of snake scales were analyzed phenetically. The goal of phenetic analysis is to estimate overall similarity (Sneath and Sokal, 1973; Voris, 1977). A variety of phenetic methods are available and there is no general agreement as to which method is superior. Accordingly, Sneath and Sokal (1973) stated that the simplest methods should be used.

The phenetic methods used here involve three steps (Voris, 1969, 1977). First, a species-by-character matrix was constructed. Second, a similarity coefficient was calculated for each possible pair of species according to the following:

$$S.C. = \frac{\text{Number of character states in common}}{\text{Total number of characters}}$$

The similarity coefficients, or matching coefficients (Sokal and Michner, 1958), were arranged in a species-by-species similarity matrix. Finally, cluster analysis was used to determine groupings of similar species.

Three basic types of cluster analysis are described by Voris (1969, 1977) and by Sneath and Sokal (1973): single linkage, complete linkage, and average linkage clustering. Alternative names include nearest neighbor method or minimum method for single linkage clustering and furthest neighbor or maximum method for complete linkage clustering. These names illustrate that single and complete linkage clustering represent two extremes-- single linkage produces the minimun number of clusters or groups and complete linkage produces the maximum number. Average linkage produces an intermediate number of clusters. The relationship among the three methods is illustrated in Figure 5 (from Voris, 1969).

Problems: Scale Study

Two categories of problems were encountered in studying snake scale surfaces: technique and specimen quality. Cole and VanDevender (1976) removed Sceloporus scales by lifting individual scales from an intact specimen with forceps-- a technique that did not work with snake scales. Experimentation led to a standard technique utilized on all snakes; i.e., cutting away a small square of skin (ca. 1 cm^2 or

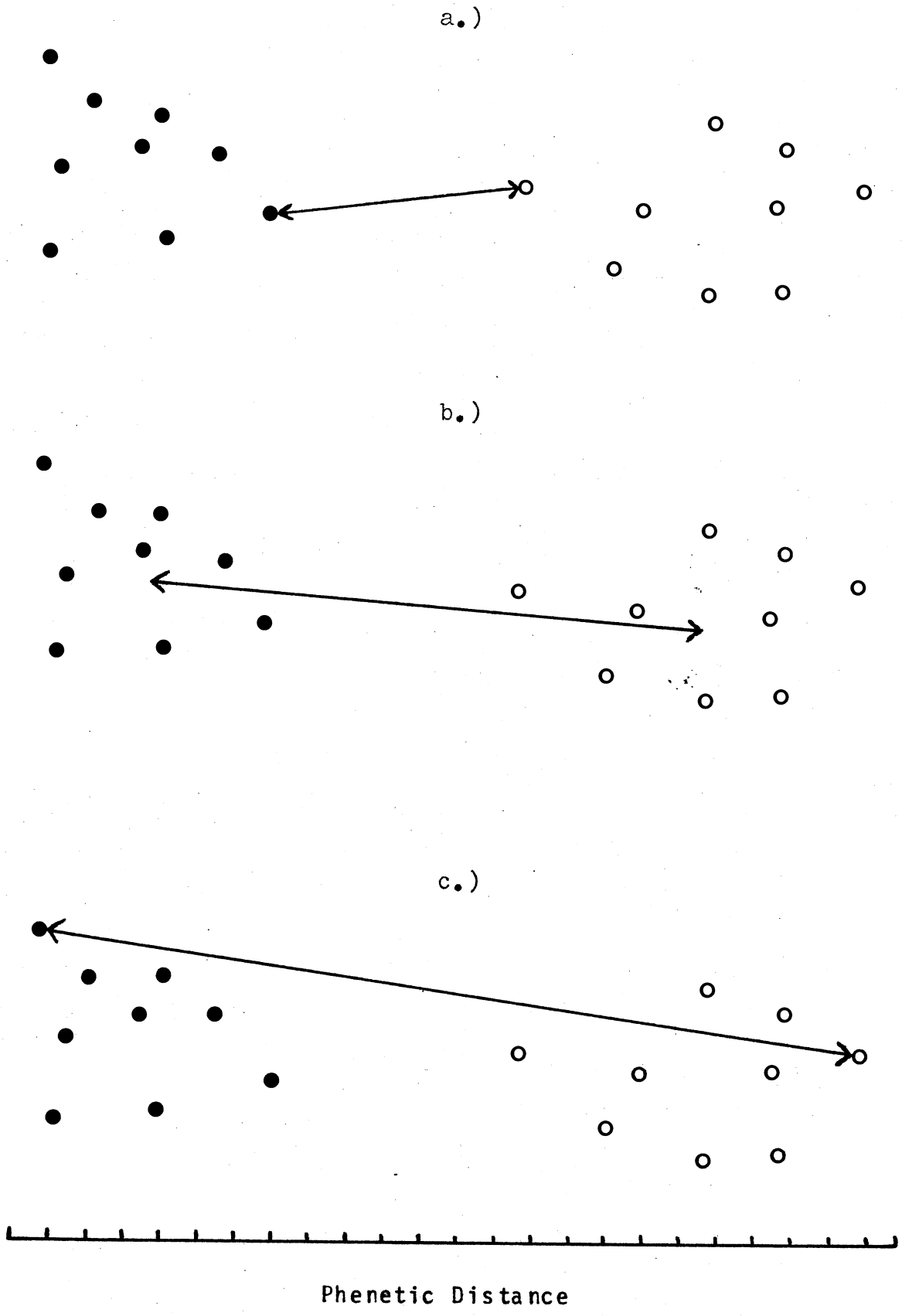
Figure 5. Methods of Cluster Analysis
(from Voris, 1969)

a.) Single Linkage Clustering

b.) Average Linkage Clustering

c.) Complete Linkage Clustering

In each case the solid dots represent the members of one already formed group, and the open circles represent members of another group. The double-headed arrow represents the similarity level of the two taxa which dictate the linking of the two groups. A phenetic distance scale is given on the horizontal axis in arbitrary units.


a.)
b.)
c.)
Phenetic Distance

less) with a razor blade. Further scale processing proceeded essentially as described by Cole and VanDevender (1976).

The second problem was more difficult. Specimen quality is variable and largely unpredictable from gross observation of a typical museum specimen. Some museum specimens are not as well preserved as hoped and have a ragged appearance. Such specimens were never used. In other specimens the "outer skin" seems to slip off the animal as soon as it is touched. This "outer skin" was not used either. The bulk of the specimens fall into neither of the above categories and look as if they should provide good quality material. Many of these typical specimens simply do not provide clean, sharp scale surfaces, even after washing the scales in a sonic bath (see Laboratory Techniques section). Unfortunately, washing away surface debris is not an adequate solution, because an obscured surface often remains. Appendix D lists all specimens observed and, as indicated, many were too obscured (Bad) to be of use.

Specimens from which sharp, clear (Good) scale preparations were obtained were compared with "Bad" specimens both grossly and under a dissecting microscope. In some cases the good specimens looked sharper at this level. Bad specimens did not always look so, however, until observed in the scanning electron microscope. Thus bad specimens took preparation time and materials causing the total project expense to be greater than a survey of the results might indicate.

Cole and VanDevender (1976) discussed variations in scale surface appearance due to variations in ecdysis cycle. Ecdysis cycle variations contribute to the problems in this study as well. There is no way to determine how recently a specimen may have shed at time of preservation. The only way to insure tissue specimens being taken from freshly exposed surfaces, is to maintain living snakes and actually monitor their ecdysis cycle. It is possible to do this, but considerable loss of resources occurs if museum specimens are not utilized.

It is significant that in this study different specimens of the same species appear to show virtually identidal surface morphologies. Therefore, unless dealing with intraspecific variations, it is probably reasonable to base descriptions on one or a few good specimens.

The nature of the shed surface is also problematic. Cole and VanDevender (1976) stated that sheds were compared to freshly exposed scales and showed essentially similar surface patterns. They noted that the shed surfaces were worn and eroded, but nevertheless were essentially the same as the new surfaces. They went on to criticize Burstein et al. (1974) for actually using erosion-produced morphology as distinctive surface features.

Shed surfaces were compared to freshly exposed surfaces from one specimen of Thamnophis marcianus (OSUR3885). As expected, the two present remarkably similar surface features (Figure 6). Thamnophis radix (OSUR598) presents

Figure 6. New vs. Old Scale Surface

a.) *Thamnophis marcianus* Scale

b.) *Thamnophis marcianus* Shed

c.) *Thamnophis radix* Scale
(with part of shed attached)

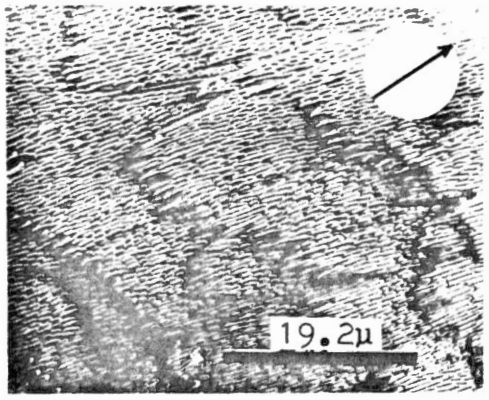
d.) *Thamnophis radix* New Scale Surface

e.) *Thamnophis radix* Old Scale Surface

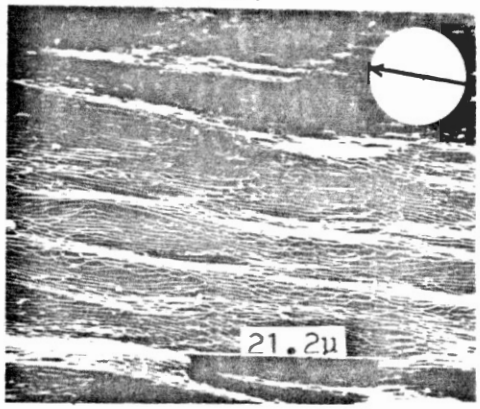
f.) *Tropidoclonion lineatum* Scale
(with old surface partially peeled away)

Arrows indicate the longitudinal axis of the scale; arrow heads point anteriorly

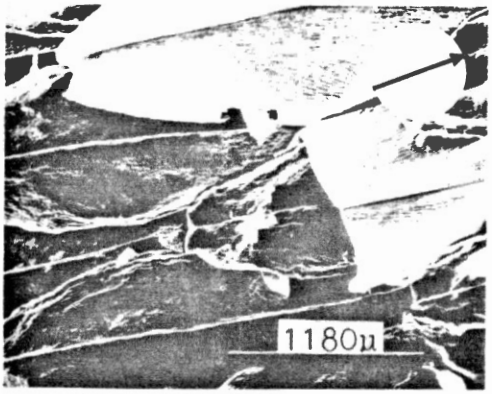
a.)



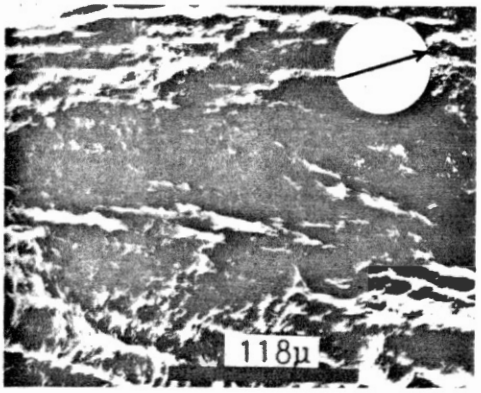
b.)



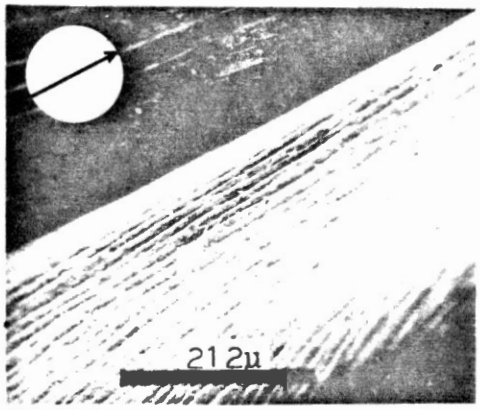
c.)



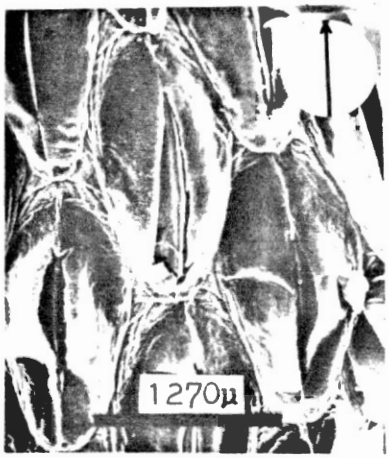
d.)



e.)



f.)



contradicting data, however. Here the outer shed is gone from most scales but still in place in a few areas. The old or outer surface is sharp and clear, but the lower and presumably new surface shows no detail at all (Figure 6). Tropidoclonion lineatum (OSUR1107) provided another opportunity to view shed and fresh scale surfaces simultaneously. In this specimen the outer layer is only partially peeled open exposing the new surface beneath. Here the situation is the reverse of that for Thamnophis radix, with a sharp clear new surface and an obscured outer surface (Figure 6). Thus the actual appearance of scales or shed surfaces must depend upon more than simple erosion, at least for preserved specimens.

CHAPTER III

RESULTS

Eyes

Microstructure

Data collected from light microscope observation of retinal sections includes percent of: visual cells, interneurons, ganglion cells, Type A cells, Type B cells, Type C cells, and Type D cells (Appendix D).

Agkistrodon. Observations of Agkistrodon contortrix and A. piscivorus basically conform to descriptions in Walls (1942) and Underwood (1970). There is no evidence to suggest that these two species show any substantial differences in visual cell types or other retinal features, although micrographs presented here (Figure 7) appear different due to differences in section thickness and staining. Using Walls' terminology of visual cell types, Type A single cones, Type B double cones, and Type C rods were all observed in Agkistrodon contortrix, with the rods being most numerous (ca. 80% of the total) (Figure 8). Type B double cones were not observed in A. piscivorus, but section thickness may account for this, since the small accessory cells are easily obscured. In general, Type C rod ellip-

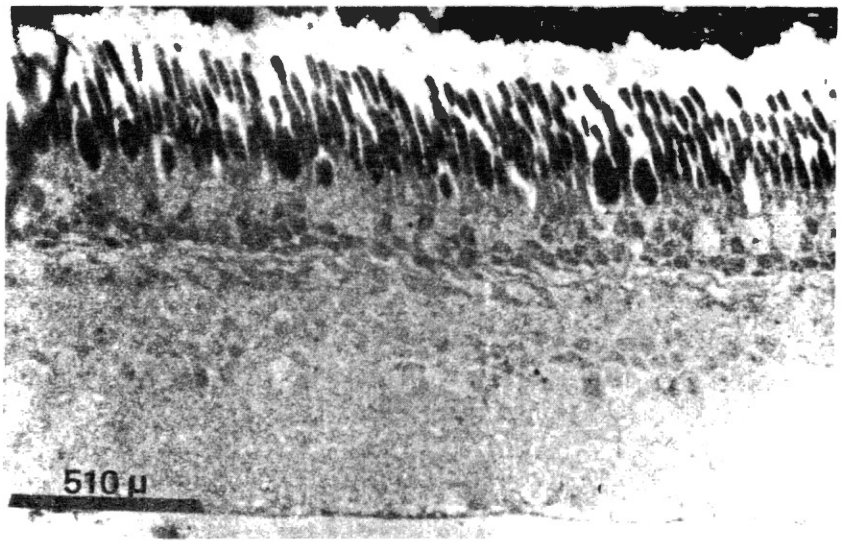
Figure 7. *Agkistrodon* Retinae

a.) *A. contortrix* Epoxy Section

b.) *A. piscivorus* Paraffin Section

Three distinct layers of cell nuclei are visible:
A = visual cells
B = interneurons
C = ganglion cells

a.)



b.)

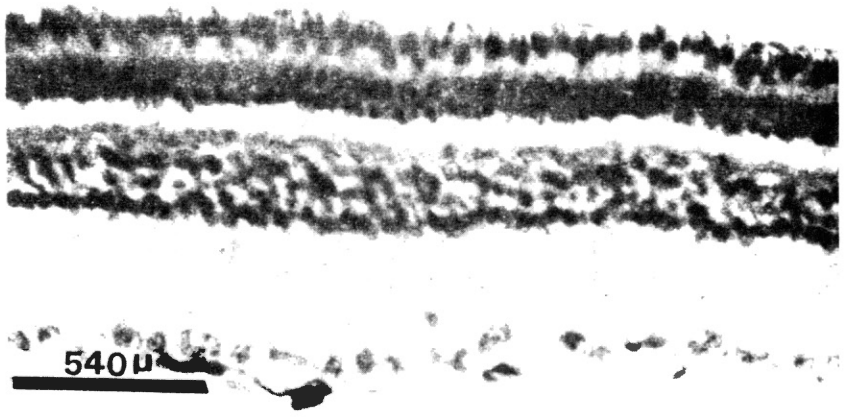


Figure 8. *Agkistrodon* Visual Cells

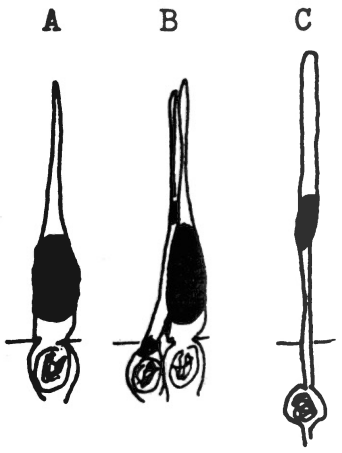
a.) Three Types Described by Walls (1942)

b.) *A. contortrix* Epoxy Section

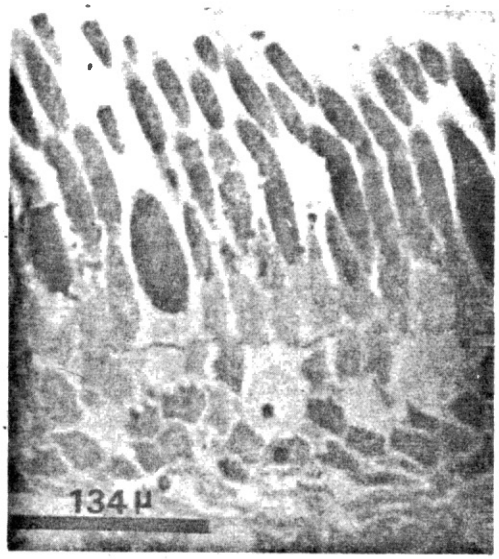
c.) *A. contortrix* Tangential Section

A = Type A cones
B = Type B double cones
C = Type C rods

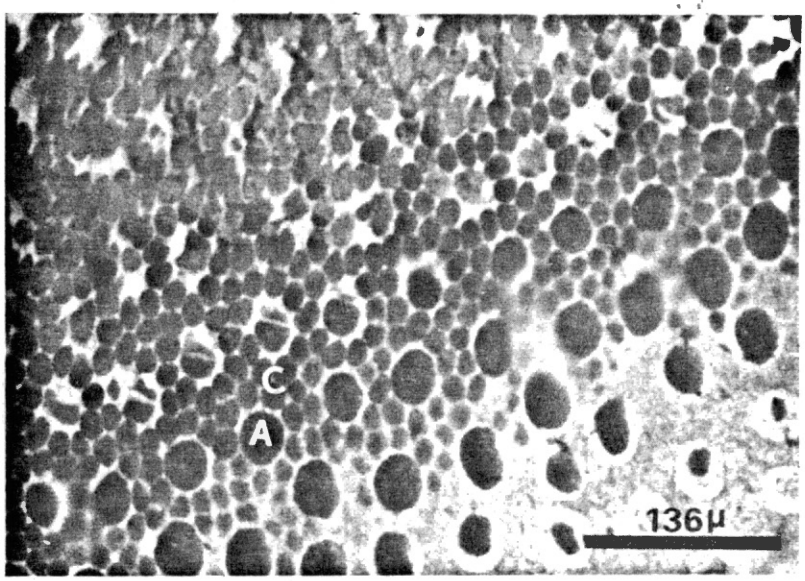
a.)



b.)



c.)



soids are located in a sclerad layer to the Type A and B cone ellipsoids. cone ellipsoids of A. piscivorus stain red in Mallory Connective Tissue Stain, in contrast to blue staining in rod ellipsoids. The red-staining regions within cone ellipsoids probably represent the "refringent body" described by Underwood (1970). Tangential sections showed that the large cones are evenly spaced. The inter-cone spaces are about twice the diameter of the smaller rod cells which surround the cones (Figure 8).

Counts of A. contortrix receptor, interneuron, and ganglion cell nuclei (50%, 45.5%, and 4%, respectively) show similar numbers of receptors and interneurons.

Farancia. The retina of Farancia was described by both Walls (1942) and Underwood (1970) as conforming to the viperine pattern as illustrated by Vipera. Vipera was reported to possess 4 visual cell types in the following frequency: rods 57%, small cones 5.5%, large single cones 29%, and double cones 8.5% (Underwood, 1970). Walls explained this pattern as a typical diurnal colubrid pattern of Type A single cones, Type B double cones, Type C single cones to which rods (Walls-Type C', Underwood-Type D) were added.

Epoxy sections of Farancia abacura (Figure 9) reveal large, prominent Type A single cones that represent some 45% of the total identified visual cells. Type B double cells are also present, but in smaller numbers, about 10% of the total. The smaller Type C and C' cells are difficult to

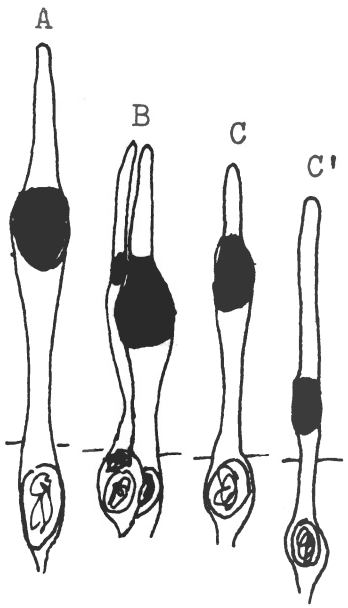
Figure 9. *Farancia* Visual Cells

a.) Four Types Described by Walls (1942)

b.) *F. abacura* Epoxy Section

A = Type A cones
B = Type B double cones
C = Type C cones
C' = Type C' rods (Type D rods in Underwood's [1970] terminology)

a.)



b.)

distinguish in these preparations. Overall, Farancia abacura has about 46% small elements (C and C' types).

Counts of receptor, interneuron, and ganglion cell nuclei (23%, 66%, and 10.5%, respectively) show interneuron cells to greatly outnumber receptors.

Nerodia. Walls (1942) and Underwood (1970) described Natrix as possessing a retina typical of diurnal colubrids. Walls specifically reported observations of Natrix natrix. Two specimens of Nerodia erythrogaster were studied here, and both possess similar retinae (Figure 10). As reported earlier (Stovall, 1975), it is often difficult to identify Type B cells in epoxy sections because the accessory member of the pair is so small it is frequently missed by a given section. For this reason it is useful to count paranuclear bodies in addition to inner-outer segments. Paranuclear bodies are unique to the accessory cells of snakes (Walls, 1942). The results of Type B uncertainties are reflected in the counts for these two specimens (Appendix D). Other retinal parameters, such as receptor and interneuron numbers, are quite similar. Type A cells dominate, representing 67% to 76% of the total in the two specimens, with smaller numbers of Type B and Type C cells. Receptor, interneuron, and ganglion cell nuclei represent ca. 13%, 77%, and 9% of the total for both specimens studied.

Nerodia fasciata shows a pattern that is very similar to that presented in Walls' preparations of Natrix natrix.

Figure 10. *Nerodia* Visual Cells

a.) Three Types Described by Walls (1942)

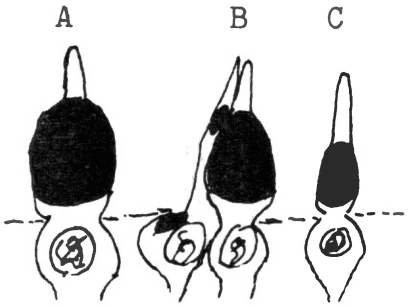
b.) *N. erythrogaster* Epoxy Section

c.) *N. fasciata* Celloidin Section

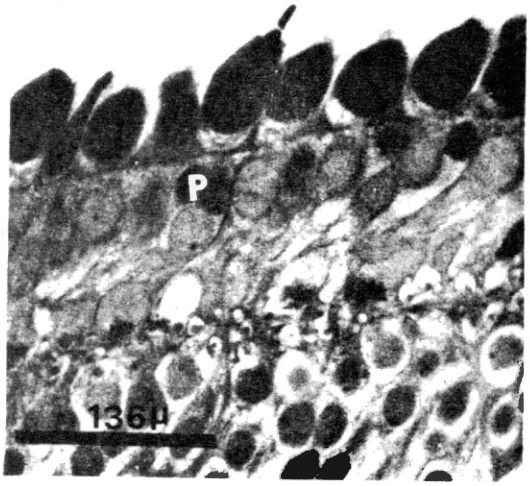
d.) *N. rhombifera* Epoxy Section

A = Type A cone
B = Type B double cones
C = Type C cone
D = Type C' cell (Type D in Underwood's [1970] terminology)
P = paranuclear body

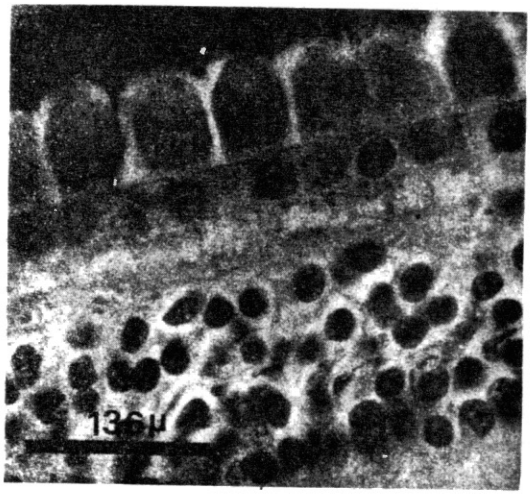
a.)



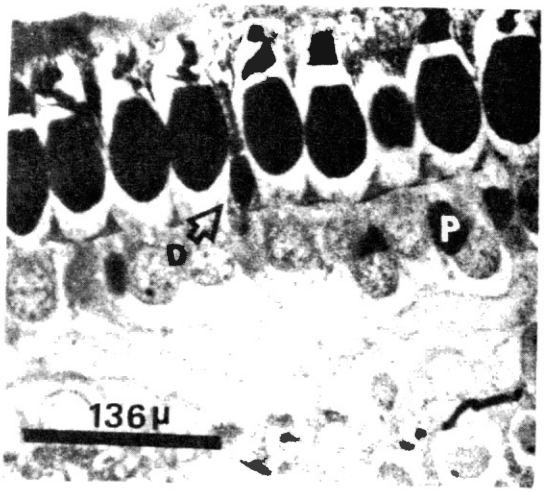
b.)



c.)



d.)



Nerodia fasciata eyes were fixed in Kolmer's fixative, embedded in celloidin, and sectioned on a rotary microtome. The section shown in Figure 10c was stained with hematoxylin and eosin. Such preparations appear quite different from the toluidine blue stained epoxy sections presented for other Nerodia specimens, but basic retinal patterns are nevertheless, quite similar. Possibly due to section thickness and/or staining variations, Type B cells were difficult to identify and the counts reflect this fact (Type A 89%, Type B 3%, Type C 8%; Appendix D). Receptor, interneuron, and ganglion cell nuclei represent 13%, 78%, and 9% of the total nuclei counted.

Nerodia rhombifera epoxy sections show a retinal pattern fundamentally similar to others of the genus (Figure 10d). Type A cells account for about 67% of the total visual cells identified, Type B cells about 17%, and the rest are smaller cells. These small cells are somewhat variable and show a range of sizes and positions. Sometimes the ellipsoids of these cells appear in line with the Type A and B ellipsoids, but only half as large; these small cells probably represent Type C cells in the Walls-Underwood nomenclature. A second row of ellipsoids, about a fourth as large as A and B ellipsoids, appears below the line of the other cells; these probably correspond to C' cells of Walls (D of Underwood) (Figure 10d, Appendix D). Receptor, interneuron, and ganglion cell nuclei are 15%, 77%, and 8% of the total nuclei counted.

Pelamis. Epoxy sections of the retina of Pelamis platurus reveal a confusing pattern (Figure 11). The ellipsoids are somewhat staggered, although the receptor nuclei are in a single row. In a typical section there are several ellipsoid profiles that are unidentifiable, yet study of other ellipsoids present reveals a typical diurnal colubrid pattern of Types A, B, and C cones in the following frequencies: 83%, 8%, and 9% (Appendix D). Hibbard and Lavergne (1972) stated that they could not identify any Type B cells and were unsure about C cell frequencies. Receptor, interneuron, and ganglion cell nuclei are 23%, 66%, and 10% of the total nuclei counted.

Regina. Epoxy sections of Regina grahami retina are similar to those of Nerodia species (Figure 11). All three visual cell types are present in frequencies similar to the other watersnakes; i.e., Type A ca. 65%, Type B ca. 17%, Type C ca. 18% (Appendix D). Receptor, interneuron, and ganglion cell nuclei are 16%, 74%, and 10% of the total nuclei counted.

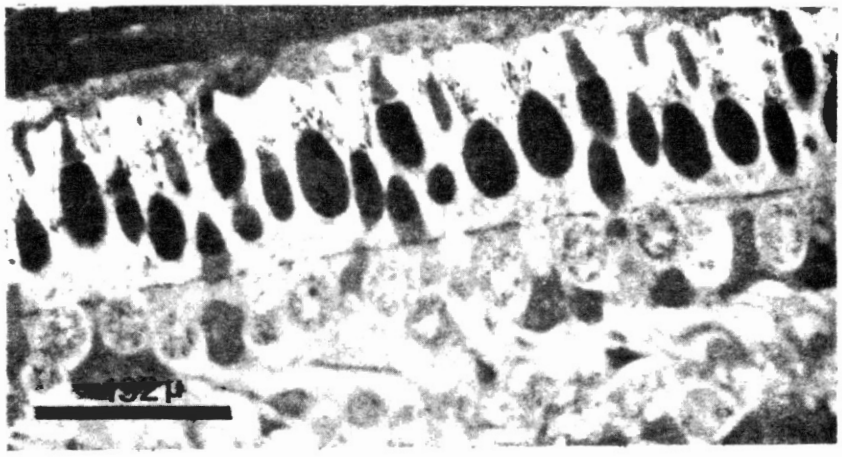
Storeria. Serial epoxy sections of the eyes of Storeria dekayi show a retina similar to other diurnal colubrids such as Coluber or Nerodia. No published studies of Storeria retinae are available for comparison to the preparations described here. Types A, B, and C cones are all present (Figure 12). The Type C cells are very rod-like, however, in being slender and elongate. Receptor, inter-

Figure 11. *Pelamis* and *Regina* Visual Cells

a.) *Pelamis platurus* Epoxy Section

b.) *Regina grahami* Epoxy Section

a.)



b.)

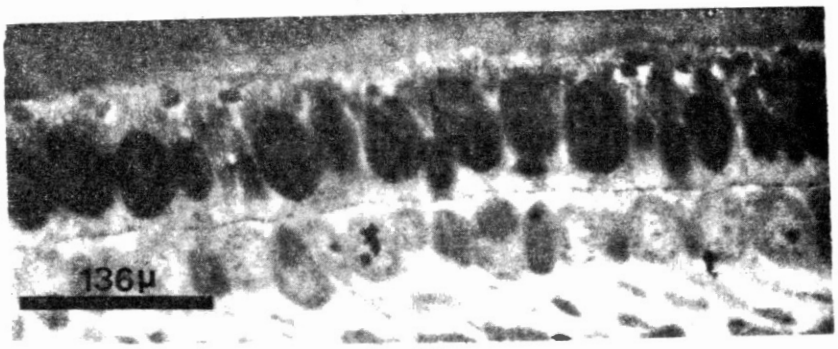


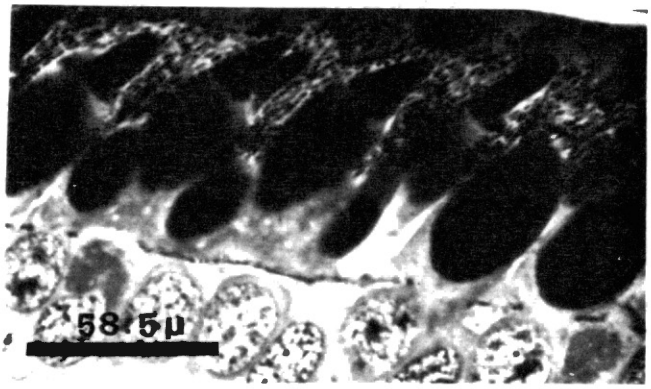
Figure 12. *Storeria* and *Thamnophis* Visual Cells

a.) *Storeria dekayi* Epoxy Section

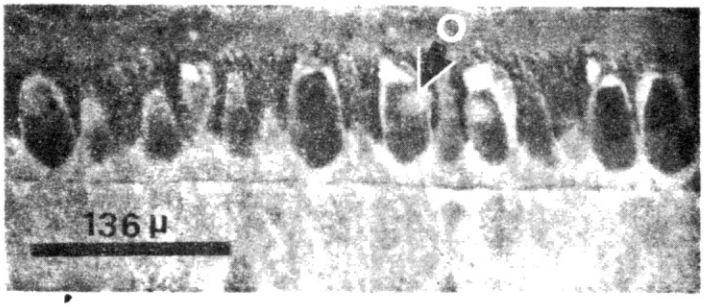
b.) *Thamnophis proximus* Epoxy Section

O = oil droplet-like structure

a.)



b.)



neuron, and ganglion cell nuclei are 12%, 79%, and 9% of the total nuclei counted (Appendix D).

Thamnophis. Epoxy sections of Thamnophis proximus retinae reveal a visual cell pattern similar to Nerodia, with Type A cells accounting for ca. 81%, Type B cells ca. 10%, and Type C cells ca. 9%. Receptor, interneuron, and ganglion cell nuclei are 12%, 80%, and 7% of the total nuclei counted (Figure 12, Appendix D).

Ultrastructure

The retinae of Storeria dekayi and Thamnophis proximus were studied in thin section via transmission electron microscopy. Although two species cannot adequately reflect all members of the Natricinae, they can provide a sample of natricine visual cell ultrastructure.

Since the retinae of diurnal colubrids have basically similar microstructures one would expect them to have similar ultrastructures as well. This expectation is verified by observations of Storeria dekayi and Thamnophis proximus. Detailed accounts of snake visual cell ultrastructure have been presented elsewhere (Underwood, 1970; Stovall, 1975, 1976a,b) and a summary presented in the introduction, so only the most striking features will be presented here.

Storeria dekayi. The pigment epithelium of other groups of vertebrates has been reported to possess inclusion

bodies called phagosomes that probably represent phagocitized outer segment lamellae (Rodieck, 1973; Young, 1976, 1977; Anderson, Fisher, and Steinberg, 1978, Hollyfield and Basinger, 1978). Phagosomes have not been previously identified in the retinal pigment epithelium of snakes, however. Phagosomes were observed in the pigment epithelium cells of Storeria dekayi (Figure 13).

The outer segment of vertebrate visual cells is believed to be attached to the inner segment of the photoreceptor by a ciliary stalk, with an associated centriole at its base (see Rodieck, 1973 for review). Although such a structure was not observed in Coluber or Hypsiglena (Stovall, 1975, 1976b), it was observed in the Type C cells and the accessory member of the double cells in Storeria (Figure 13).

Ellipsoids of snakes, like those of other vertebrates, consist of mitochondrial aggregates (Underwood, 1970; Stovall, 1975, 1976b). The mitochondria are highly modified or even absent in the center of snake ellipsoids, however. As shown in Figure 13, Storeria ellipsoids contain a central region composed of aggregates of electron dense or electron lucid vesicles. This unusual structural arrangement is probably responsible for light microscope descriptions of densely staining refringent bodies inside snake ellipsoids (Underwood, 1970).

Thamnophis proximus. Ellipsoids of Thamnophis proximus

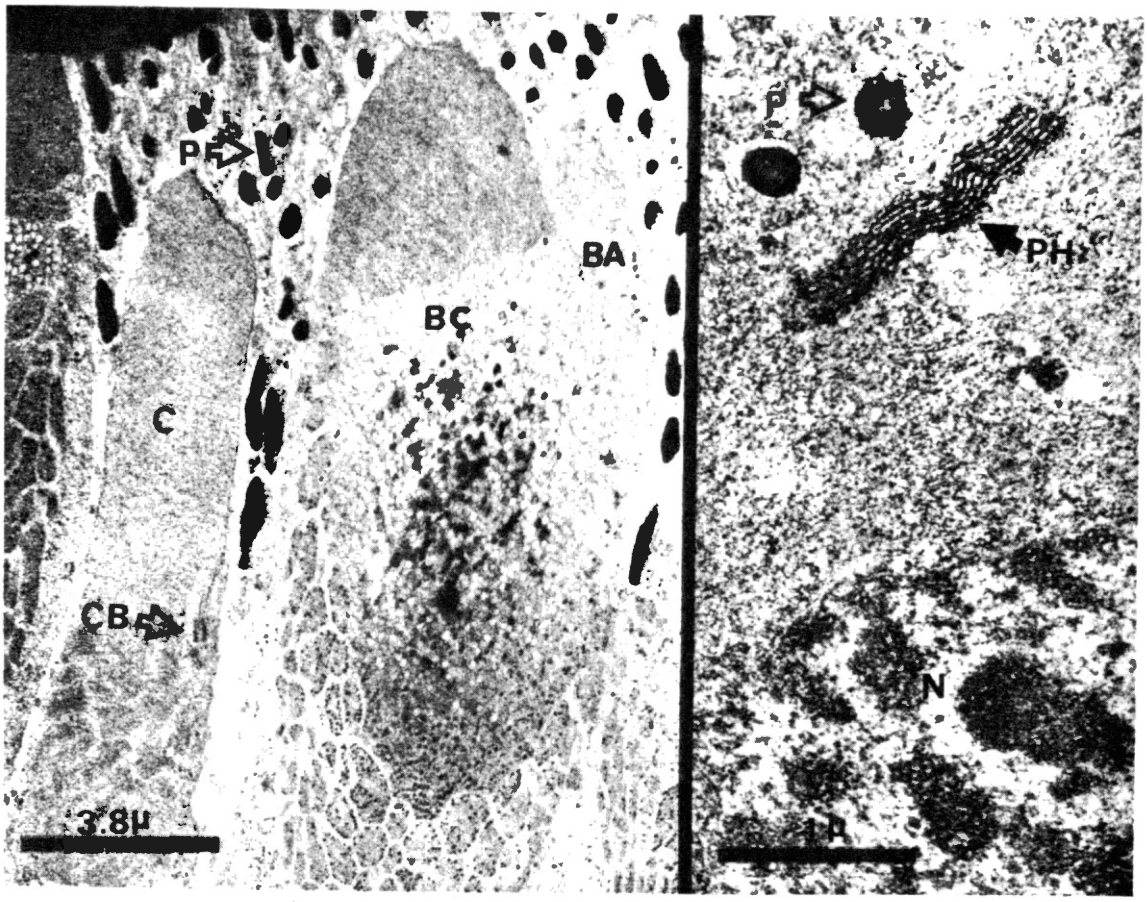
Figure 13. *Storeria dekayi* Retinal Ultrastructure

a.) Type B and Type C cones

b.) Phagosome in Pigment Epithelium Cell

BA = Type B accessory cell
BC = Type B chief cell
C = Type C cell
CB = ciliary body
N = nucleus
P = pigment granules
PH = phagosome in pigment epithelium

a.) b.)



are most striking in appearance. The periphery of the ellipsoid is clearly composed of mitochondria, which generally contain electron dense granules. The central region consists of a dense aggregate of electron dense granules that show a range of densities (Figure 14).

An oil droplet-like body was observed at the distal end of the ellipsoid in some visual cells (Figure 15). Oil droplets have not been reported from visual cells of other snakes (Walls, 1942; Underwood, 1970), however, the structures observed in Thamnophis proximus resemble published electron micrographs of oil droplets in other vertebrates (Pedler and Tansley, 1963; Young, 1977). The droplet-like structures may represent fixation artifacts, since obvious artifacts such as disrupted membranes were observed in surrounding regions. These droplets appear too large and consistent in location to represent typical fixation artifacts I have observed elsewhere, however.

The ciliary stalk with basal body and associated centriole is well demonstrated in Thamnophis accessory cells (Figure 14). Ciliary structures were not observed in other cell types. The ellipsoid region of the accessory cells never contains the high degree of organization found in other classes of cells. The accessory cell ellipsoid is composed of a few rather loosely packed mitochondria, with no central body and rare intra-mitochondrial granules.

Figure 14. *Thamnophis proximus* Type B Double Cell

C = ciliary body in accessory cell
G = granule in chief cell ellipsoid
O = outer segment
P = pigment granule

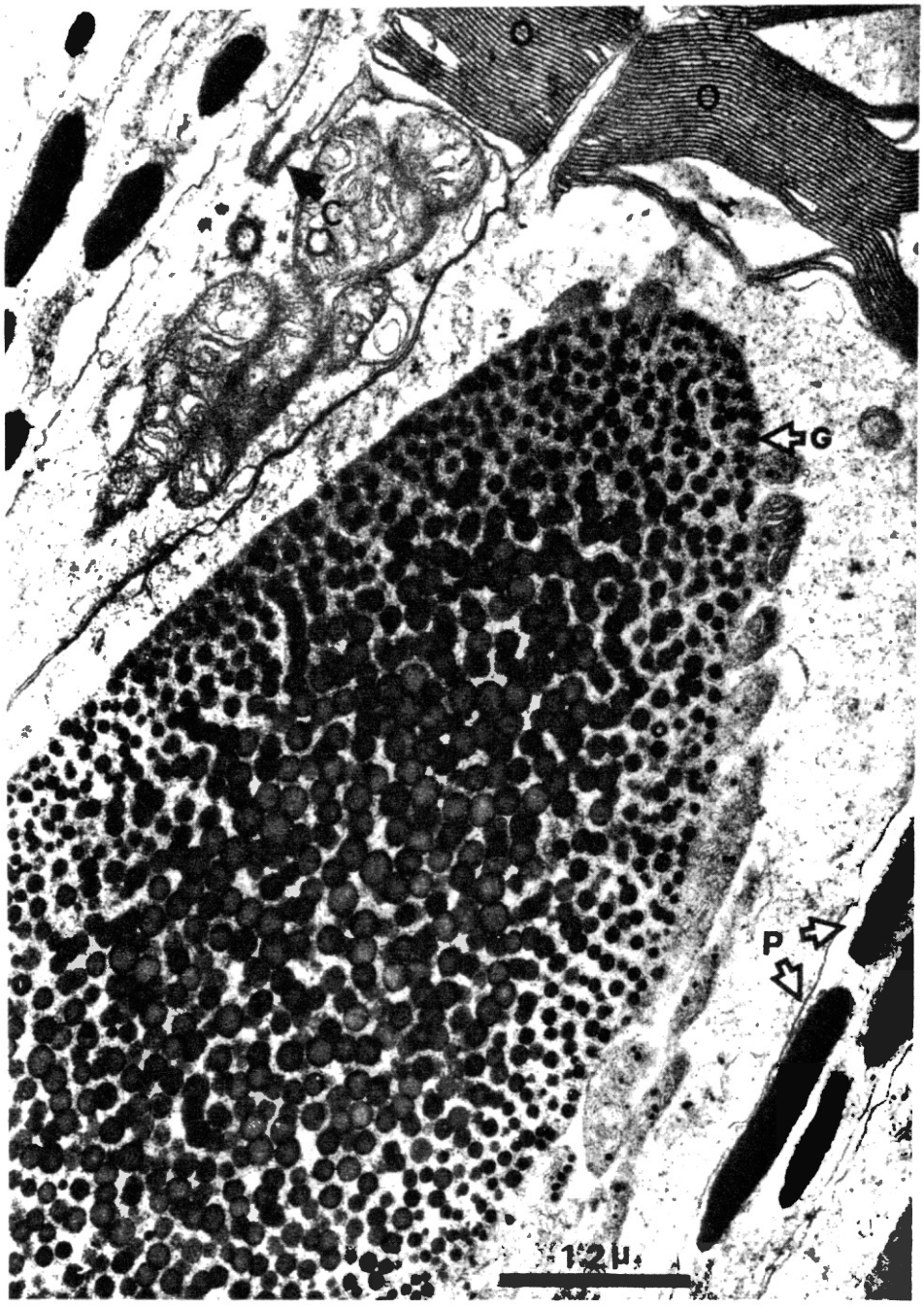
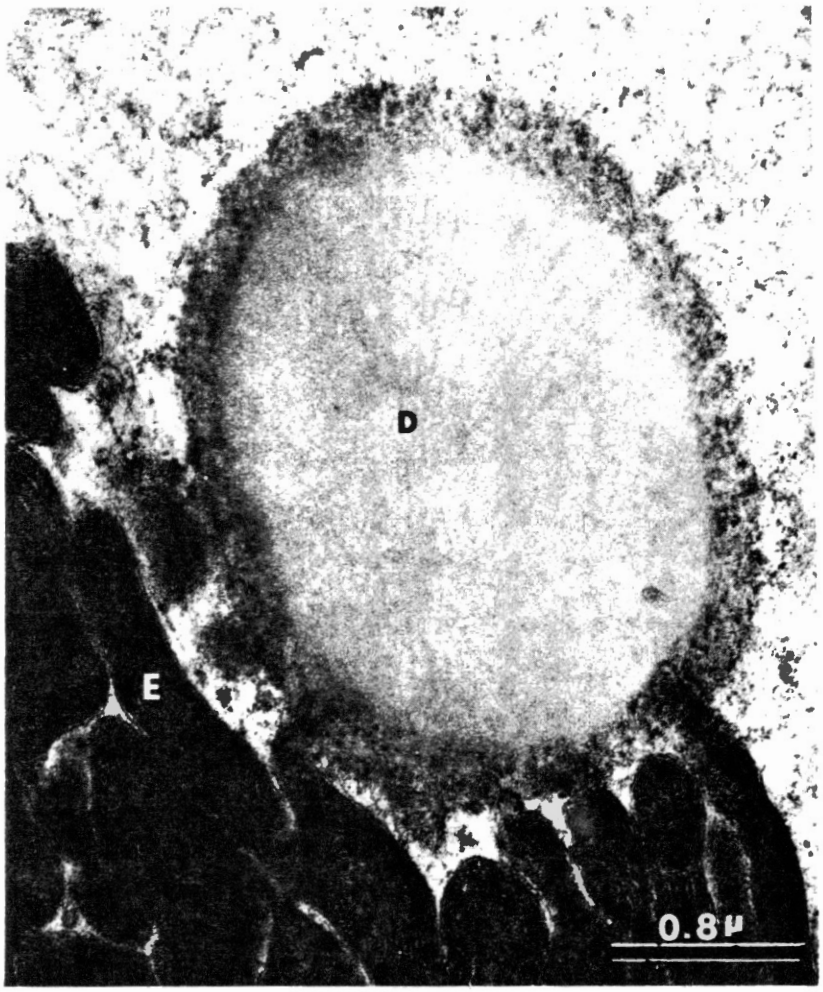

O
O
C
G
P
1.2μ

Figure 15. *Thamnophis proximus* Droplet-like Structure

D = droplet
E = ellipsoidal mitochondria


D
E
0.8μ

Scales

Snake scale surfaces differ markedly from Sceloporus scales as described by Cole and VanDevender (1976). In Sceloporus and Uta (Stovall, in prep.), both iguanids, each scale appears uniform and well defined at low magnification, but higher magnifications reveal a pattern of distinct plates. According to Stewart and Daniel (1973, 1975) and Cole and VanDevender (1976) these plates represent individual oberhautchen cells. No such repeating "cellular" units are clearly visible on snake scales. Rather, each snake scale seems to be the unit of structure. Scales of Sceloporus and Uta also show distinctive depressions, perhaps sensory pits, in their surfaces (Cole and VanDevender, 1976; Stovall, in prep.). No such distinctive pits were observed in dorsal, midbody snake scales (see discussion of pits in Agkistrodon and Nerodia, however).

Scale Characters and Terminology

In order to facilitate comparison of scale morphologies among the species studied a set of eight characters was selected (Table III). Each of these characters is defined and specific states or conditions used to categorize scale morphologies are listed in an illustrated glossary of scale characters (Appendix E). A species-by-species comparison produced a matrix of character states (Table IV). Of the 24 species studied, analyses were performed only on those 19

TABLE III

SCALE CHARACTER STATES

Character Number	Character Name	Character State
1	Overall shape	1-1 Agkistrodon-like 1-2 Farancia-like 1-3 Nerodia-like
2	Central keel	2-1 Present 2-2 Absent
3	Apical pits	3-1 Present 3-2 Absent
4	Longitudinal ridges	4-1 Present 4-2 Absent
5	Perpendicular spikes	5-1 Spikes interdigitate 5-2 Spikes overlap 5-3 Absent
6	Inter-ridge lattice	6-1 Present 6-2 Absent
7	Polygonal units	7-1 Present 7-2 Absent
8	Anastomosing ridges	8-1 Present 8-2 Absent

TABLE IV

SPECIES-BY-CHARACTER DATA MATRIX

Species Number	Species Name	Character Number 1	2	3	4	5	6	7	8
1	Agkistrodon piscivorus	1	1	1	1	3	2	2	2
2	Farancia abacura	2	2	2	2	1	2	2	2
3	Nerodia fasciata	3	1	1	1	3	1	2	2
4	Nerodia rhombifera	3	1	1	1	3	1	1	2
5	Nerodia sipedon	3	1	2	1	3	1	2	2
6	Regina alleni	2	2	2	1	2	2	2	2
7	Regina rigida	3	1	2	1	2	2	2	2
8	Regina septemvittata	3	1	2	1	3	1	2	2
9	Storeria dekayi	3	1	2	1	3	1	2	1
10	Storeria occipitomaculata	3	1	2	1	3	1	2	1
11	Thamnophis butleri	3	1	2	1	3	1	2	2
12	Thamnophis elegans	3	1	2	1	3	1	1	2
13	Thamnophis eques	3	1	2	1	3	2	2	1
14	Thamnophis marcianus	3	1	2	1	3	2	2	1
15	Thamnophis proximus	3	1	2	1	3	1	2	1
16	Thamnophis radix	3	1	2	1	3	1	1	2
17	Thamnophis sauritus	3	1	2	1	3	1	1	2
18	Thamnophis sirtalis	3	1	2	1	3	2	1	2
19	Tropidoclonion lineatum	3	1	2	1	3	2	2	1

which showed unobscured scale surfaces (Appendix A).

Species Accounts

Agkistrodon contortrix. The dorsal midbody scales of Agkistrodon contortrix are roughly rhomboidal in shape when flattened, although they are usually convexly arched outward in situ and therefore the sides may appear more constant in width over the middle and basal (anterior) portion. The base is slightly convex or arched outward anteriorly and may be covered by the apical portions of overlapping scales when the skin is not stretched. The apical (posterior) aspect tapers to a bluntly rounded tip. The most conspicuous features other than shape are (1) the central longitudinal keel and (2) two apical "pits" on either side of the keel about 1/3 the scale length from the apical margin. All these features may be observed at low magnifications; e.g., ca. 30 times or less (Figure 16).

Higher magnification reveals a system of longitudinal ridges present over the scale's surface, although only one of the three specimens studied (OSUR1099) showed this pattern clearly (Figure 16). In the other specimens the pattern is obscured. At magnifications of 300 times the apical pits appear as shallow disc-shaped depressions or concavities (Figure 16).

At similar magnifications the longitudinal ridges appear to be discontinuous but do not branch. No additional structures or pattern becomes apparent at magnifications

Figure 16. Agkistrodon Scales

a.) A. contortrix Low Magnification Overview

b.) A. contortrix High Magnification Surface Pattern

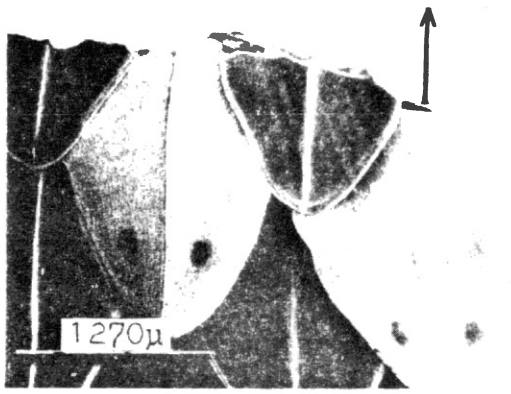
c.) A. piscivorus Low Magnification Overview

d.) A. piscivorus Moderate Magnification (apical pit in left center)

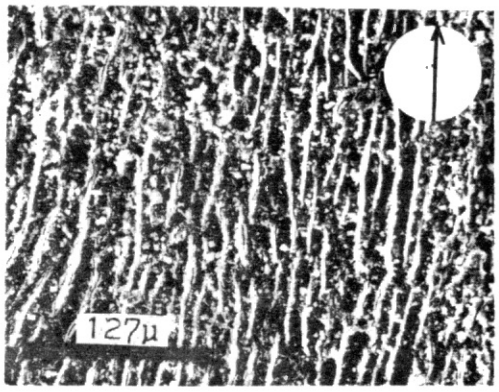
e.) A. piscivorus High Magnification Surface Pattern (central keel crosses photograph horizontally)

f.) A. piscivorus High Magnification Central Keel Flank

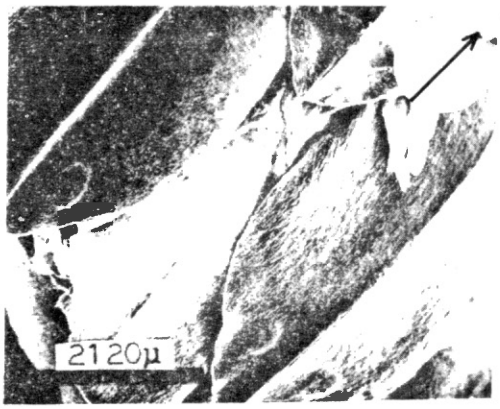
a.)



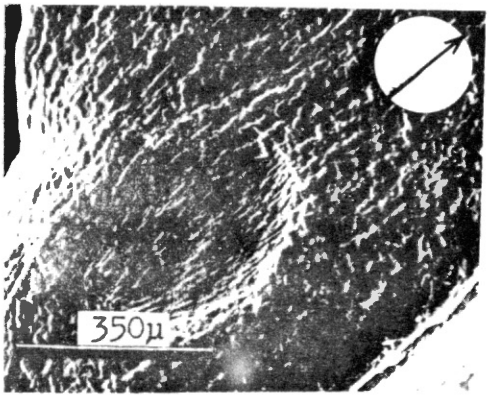
b.)



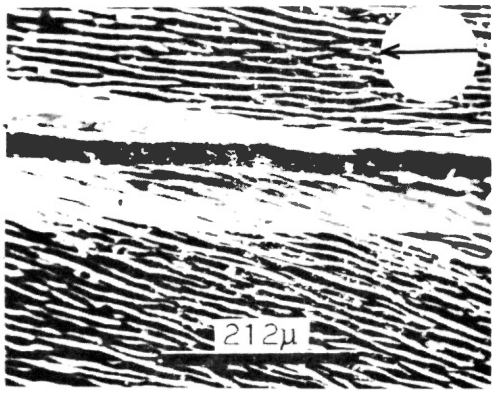
c.)



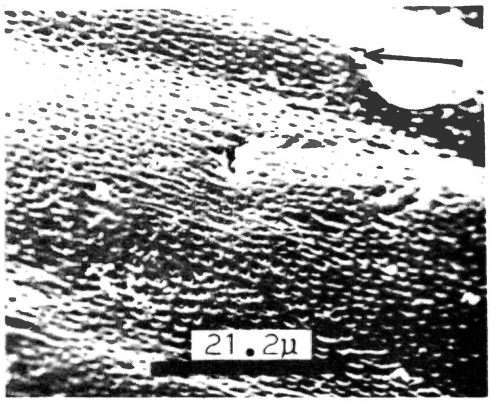
d.)



e.)



f.)



higher than 300 times. The presence of the ridges is suggested even in 30X view of specimen OSUR1009, although not in the other specimens.

Agkistrodon piscivorus. At low magnifications, dorsal midbody scales of Agkistrodon piscivorus are essentially undistinguishable from A. contortrix (Figure 16). Only one of the two specimens examined, RS89, shows detail. A pattern of longitudinal ridges is suggested at low magnifications and is confirmed at higher magnifications. In this species the ridges appear as thin plates that project from the scale forming rather deep troughs between adjacent ridges (Figure 16). Slight branching may also occur among the ridges. This specimen of A. piscivorus (RS89) appears cleaner and more well defined than OSUR1099, Agkistrodon contortrix; this may account for the differences in appearance of the ridges.

At 200X, the central keel appears to be flanked by amorphous material that may be left behind from the previous shed. Higher magnification (2000X) shows this amorphous flanking material to possess a smooth surface interrupted by numerous irregular pits (Figure 16). This region is similar in appearance to Burstein et al.'s (1974) Grade 1 pit-spinule scale pattern of Sceloporus.

Farancia abacura. Dorsal midbody scales of Farancia abacura are very smooth, shiny, and iridescent (Conant, 1975). The scales are non-keeled and distinctly rhomboidal

in shape with bluntly tapered apical margins. No pits or other conspicuous features are visible at low magnfications (Figure 17). At magnifications of 2000X or more an intricate pattern is present in both specimens studied (Figure 17). The surface is covered by horizontal rows or units (i.e., oriented perpendicular to the long axis of the scale) from which spike-like projections extend posteriorly. The spikes of one row closely interdigitate with indentations in the adjacent rows. The actual surface of the horizontal units is smooth but with irregularly distributed pits, which are very small at 2000X.

Nerodia cyclopion. At low magnifications the dorsal midbody scales of Nerodia cyclopion are relatively elongate with almost straight sides. The anterior margin is slightly recurved. Posteriorly the scales taper to a rounded apex about 1/4 as wide as the middle portion of the scale. The scales are strongly keeled and somewhat folded at the keel to produce an inverted V-shaped profile with the keel at the apex of the V. The central keel extends longitudinally the entire length of the scale from base to apex (Figure 18). No further details are apparent on specimen 1996, but specimen NELS5229 shows two additional features: light longitudinal striations or ridges and apical pits. Both ridges and pits are similar in appearance to those of Agkistrodon scales at low magnification. Higher magnification reveals an obscured pattern of longitudinal ridges

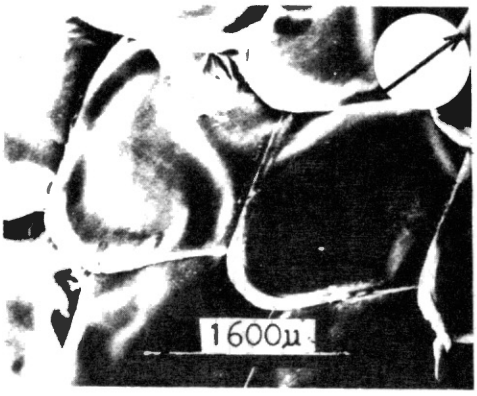
Figure 17. *Farancia abacura* Scales

a.) Low Magnification Overview

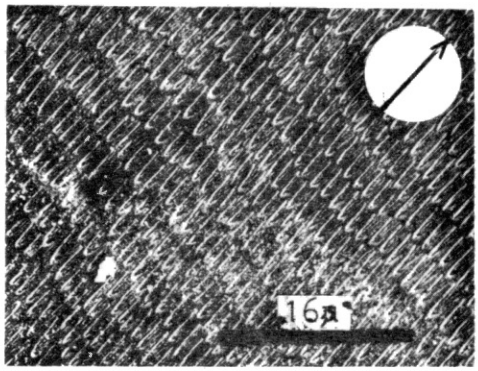
b.) High Magnification Surface Pattern (OSUR3891)

c.) High Magnification Surface Pattern (OSUR2017)

a.)



b.)



c.)

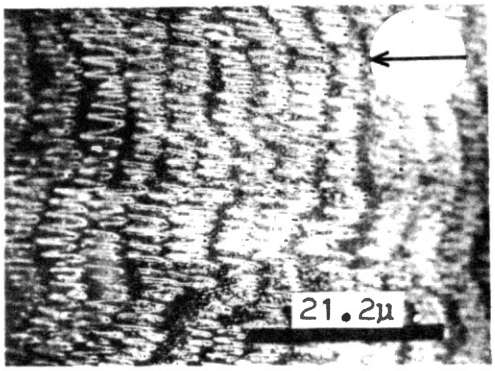


Figure 18. *Nerodia* Scales

a.) *N. cyclopion* Low Magnification Overview

b.) *N. cyclopion* Moderate Magnification Surface Pattern

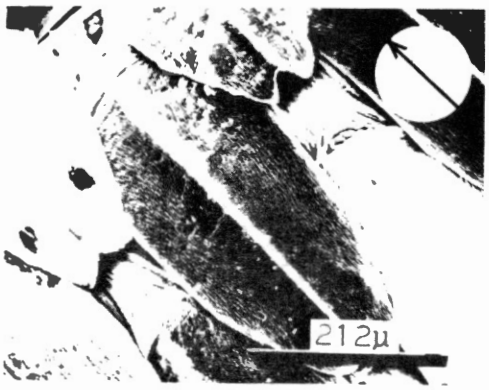
c.) *N. erythrogaster* Moderate Magnification Surface Pattern

d.) *N. fasciata* High Magnification Surface Pattern

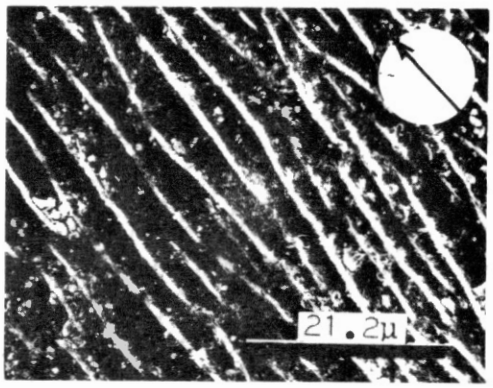
e.) *N. rhombifera* Moderate Magnification Surface Pattern (dark spot left of center is apical pit)

f.) *N. rhombifera* High Magnification Surface Pattern

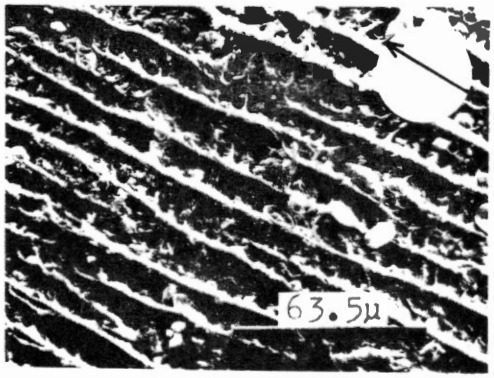
a.)



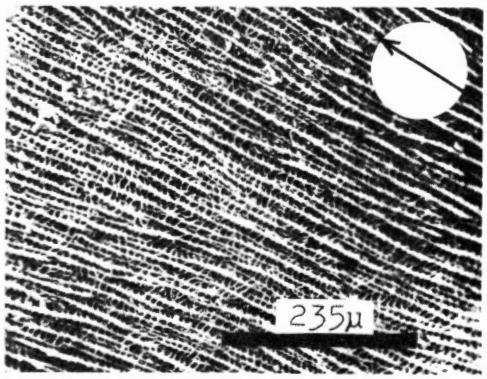
b.)



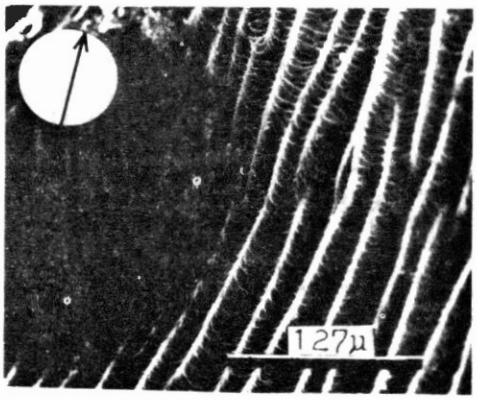
c.)



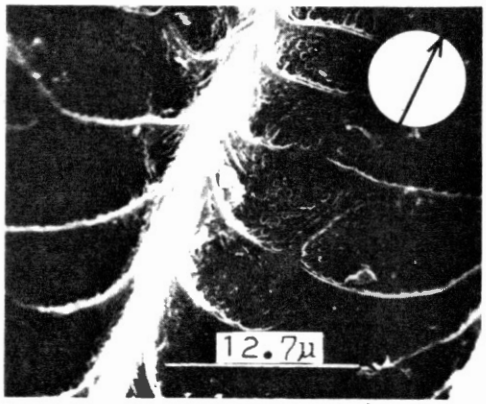
d.)



e.)



f.)



separated by rather broad troughs. No substructure can be observed in these troughs. The scales appear covered by a coating or film (Figure 18).

Nerodia erythrogaster. Low magnification observation of Nerodia erythrogaster reveals scales similar to those described for N. cyclopion except that apical pits are not observed on any of the four specimens studied. Higher magnification of specimens OSUR2677 and OSUR3145 reveals an obscured pattern of longitudinal ridges alternating with broad troughs. Specimens OSUR3475 and OSUR3031 are partially free of the obscuring covering and higher magnification reveals a definite substructure within the troughs. Troughs are filled with a lattice-work forming irregular rectangular compartments somewhat similar to the scale surfaces of Sceloporus described by Burstein et al. (1974) as deep cell surface (Figure 18).

Nerodia fasciata. Low magnification reveals Nerodia fasciata scales similar to those of N. erythrogaster in overall appearance. Specimens OSUR109, OSUR188, and OSUR-187 are obscured and show little more than gross shape and central keel. Specimen OSUR112 shows, at low magnifications, apical pits, longitudinal ridges, and an indication of some lattice-work in the troughs. Higher magnfication (1100X) shows a pattern similar to that described for N. erythrogaster; i.e., a partially obscured lattice-work. The lattice-work is formed by distinct cross walls that

extend from ridge to ridge across the surface of the trough. Much relief is observed between the cross walls and the scale surfaces surrounding them. At higher magnification the longitudinal ridges seem to arise and disappear randomly in the pattern of the lattice-work (Figure 18).

Nerodia rhombifera. Three specimens of Nerodia rhombifera show distinctly different aspects of an essentially similar pattern. All three show a gross scale morphology similar to N. erythrogaster and N. fasciata. Scales are elongate with central keel, longitudinal ridges, and apical pits. The sequence OSUR3866, OSUR3867, and OSUR3664 provides a range of structure that is suggestive of the lattice-work in the inter-ridge troughs. This lattice-work pattern is largely obscured in 3866 but becomes clearer in 3867 and is sharply defined in 3664 (Figure 18). As was true for N. fasciata, the longitudinal ridges of N. rhombifera arise and disappear randomly in the lattice-work and cross bridges connect across the troughs to adjacent ridges. The cross bridges are suggestive of cell margins and may be comparable to cell margins described for Sceloporus (Burstein et al., 1974). Still higher magnification (3000X) reveals an interesting relationship between the cross bridges and the longitudinal ridges. The cross bridges converge into the ridges in chevron-like fashion to form a striking geometric arrangement (Figure 18). The scale surfaces between the cross bridges are smooth and marked

with irregular holes or pits similar to those observed on the keel flanks of Agkistrodon piscivorus scales.

Nerodia sipedon. At low magnification Nerodia sipedon scales are similar to the Nerodia described above. Apical pits were not observed (Figure 19). A range of specimen quality from essentially no pattern (OSUR993, OSUR990) to a distinct pattern on one specimen (OSUR491) was observed. In OSUR491 the pattern clearly resembles that described for N. rhombifera. Cross bridges form chevron-like processes that converge and overlap to form the longitudinal ridges visible at lower magnification. An interesting feature found on OSUR491 may explain the pattern obscurity present on so many different specimens. Entire scales were removed along with underlying tissue, and as always, care was taken not to damage the outer surface during processing. The scales appear to be covered by an old surface or slough that is partially torn away over the central portion of the scale (Figure 19). Simultaneous observation of the old surface and new surface of the scales is thus possible (Figure 19). Old surfaces show a strongly obscured or "film-coated" pattern, whereas new surfaces underneath show a sharp clear pattern (Figure 19).

Regina alleni. At low magnification the scales of Regina alleni are similar in appearance to those of Farancia abacura; i.e., they are rhomboidal, smooth, non-keeled, with bluntly tapering apical margins (Figure 19). No pits are

Figure 19. *Nerodia sipedon* and *Regina alleni* Scales

a.) *N. sipedon* Low Magnification Overview (outer surface peeled away in center)

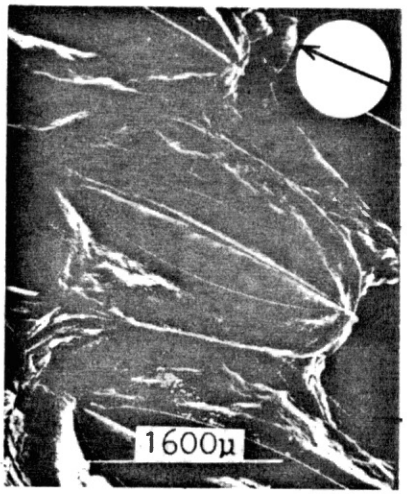
b.) *N. sipedon* High Magnification Surface Pattern (on new scale surface)

c.) *R. alleni* Low Magnification Overview

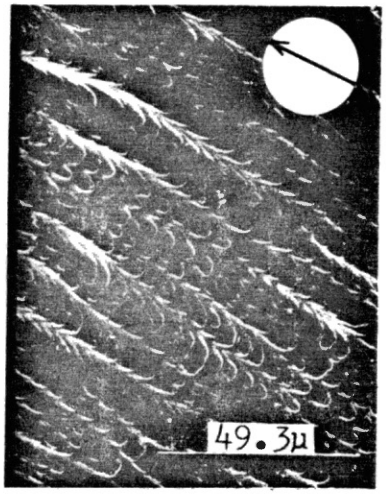
d.) *R. alleni* Moderate Magnification Surface Pattern

e.) *R. alleni* High Magnification Surface Pattern

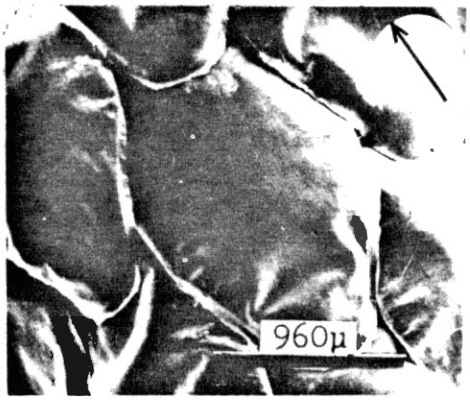
a.)



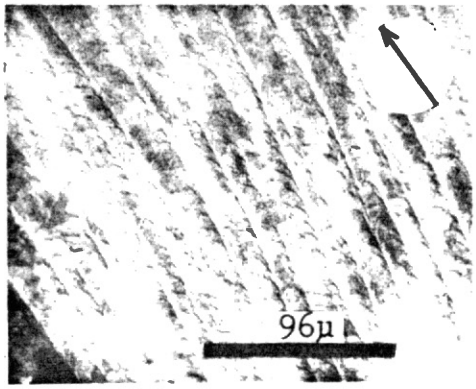
b.)



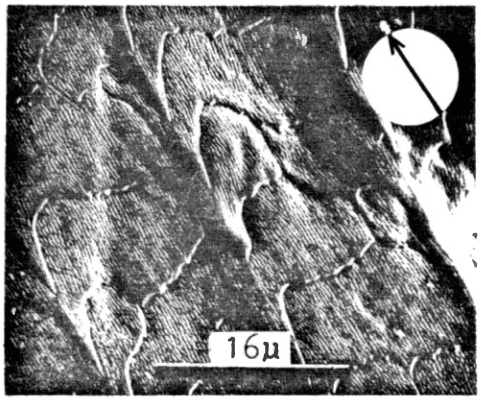
c.)



d.)



e.)



evident, but inconspicuous longitudinal striations are present.

Higher magnification shows this longitudinal striation to consist of horizontal rows of spiked units whose anterior margin is covered over by the posterior spikes of the next row (Figure 19). The posterior, visible portion of each unit consists of a sharply projecting spine or spike flanked by rather deeply emarginate borders. Each horizontal row is spatially aligned with the other rows forming longitudinal arrays of spines, which give rise to the longitudinal striations visible at lower magnification

Regina grahami. Low magnification observation shows that Regina grahami dorsal midbody scales are similar in gross appearance to those of Nerodia species, but lack apical pits. Both specimens studied show an obscured pattern at higher magnifications. Due to the apparent "coating" the inter-ridge troughs appear essentially amorphous.

Regina rigida. Low magnification observation of dorsal midbody scales of Regina rigida shows scales similar in gross morphology to R. grahami. At moderate magnifications longitudinal ridges arise and disappear randomly and a pattern is barely visible on the surfaces between the ridges (Figure 20). Higher magnification (2000X) shows a distinct pattern that somewhat resembles R. alleni at similar magnification. There are horizontal rows of overlapping spines. In contrast to the pattern of R. alleni, the

Figure 20. *Regina rigida* and *Regina septemvittata* Scales

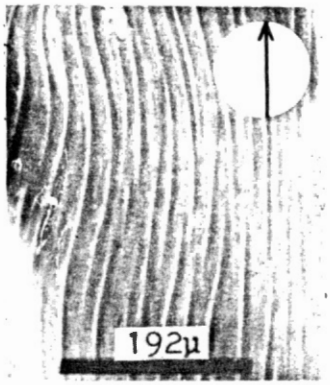
a.) *R. rigida* Moderate Magnification Surface Pattern

b.) *R. rigida* High Magnification Surface Pattern

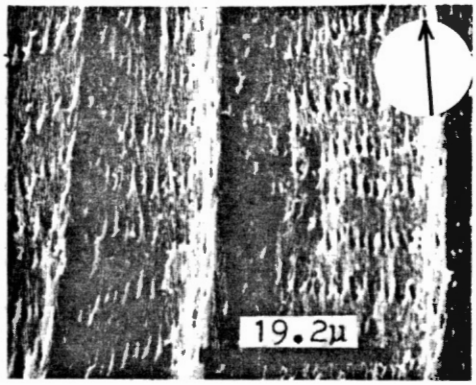
c.) *R. septemvittata* Low Magnification Overview

d.) *R. septemvittata* High Magnification Surface Pattern

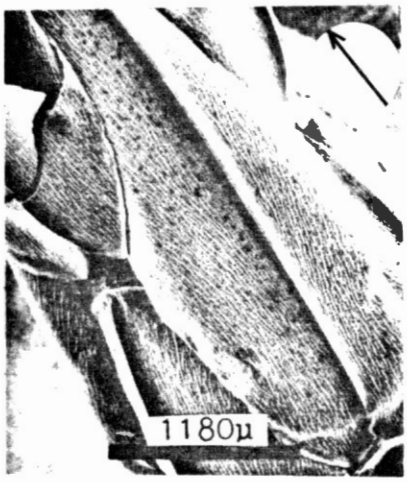
a.)



b.)



c.)



d.)

spines project posteriorly and overlap in trough as well as ridge regions. So that one sees a rather consistent pattern of sharply pointed spines that are larger at the upfolded ridges, but elsewise similar across the surface of the scale (Figure 20).

Regina septemvittata. Three specimens of Regina septemvittata show low magnification features quite similar to those of Nerodia species; e.g., overall shape, longitudinal ridges, elongate shape, central keel (Figure 20). Apical pits are absent on all three specimens. One specimen (OSUR-895) shows an obscured surface at higher magnfication, but the other two show clear, sharp patterns, distinctly similar in appearance to the Nerodia species described above and not nearly as similar to the patterns described for Regina alleni and R. rigida. The surface pattern of R. septemvittata scales consists of longitudinal ridges that arise and disappear randomly between which cross bridges form a lattice work of compartment-like units. The compartments or spaces between cross bridges are irregular in shape and size and rather deeply recessed from the surface of the cross bridges and ridges (Figure 20). As is the case for Nerodia, the cross bridges give rise to the longitudinal ridges via chevron-like extensions that overlap each other to form a continuous ridge at right angles to the ridges proper.

Storeria dekayi. Dorsal midbody scales of Storeria dekayi are similar in gross morphology to those typical of

Nerodia. Standard features are elongate shape, central keel, and longitudinal ridges or striations. At higher magnification there is departure from Nerodia or any other pattern previously described. The longitudinal ridges arise and disappear randomly from a network of thin irregularly arranged ridges. The ridges anastomose and contrast sharply with the recessed scale surface (Figure 21). Longitudinal ridges appear to consist of several of the small ridges tightly aligned together. There are no chevrons or rows of spines as observed on Nerodia or Regina scale surfaces. Of the three specimens studied, one shows virtually no pattern, but the other two clearly show the pattern.

Storeria occipitomaculata. At low magnification Storeria occipitomaculata presents a scale surface morphology virtually identical to that of S. dekayi. Neither exhibit apical pits, unlike Nerodia. Higher magnification of specimen OSUR1206 clearly shows a surface pattern similar to that observed on S. dekayi (Figure 21). Here the pattern is distinct and the small ridges contrast sharply with the recessed scale surface. The longitudinal ridges, visible at very low magnifications, appear to be longitudinal aggregates of the smaller ridges which anastomose to form a fine network between the longitudinal ridges (Figure 21).

Thamnophis butleri. Low magnification of dorsal midbody scales of Thamnophis butleri shows typical Nerodia-like shape and morphology (Figure 21). No pits are present.

Figure 21. *Storeria* and *Thamnophis butleri* Scales

a.) *S. dekayi* Moderate Magnification Surface Pattern

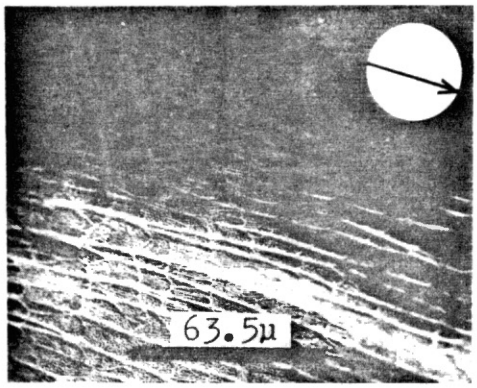
b.) *S. occipitomaculata* Moderate Magnification Surface Pattern

c.) *S. occipitomaculata* High Magnification Surface Pattern

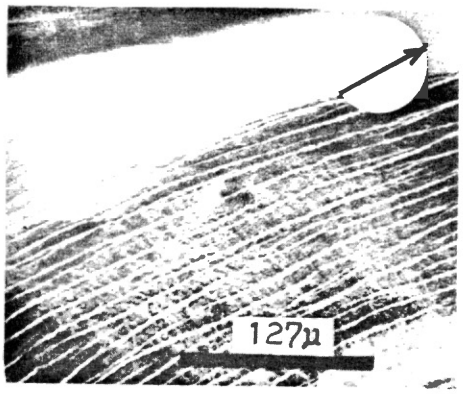
d.) *T. butleri* Low Magnification Overview

e.) *T. butleri* High Magnification Surface Pattern

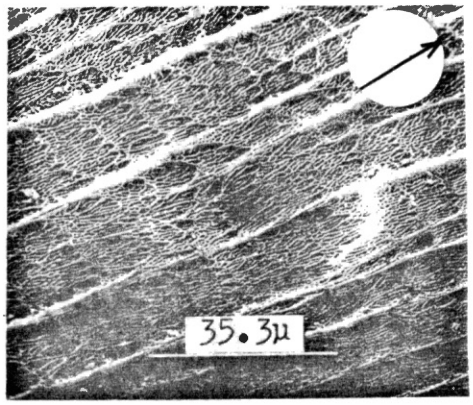
a.)



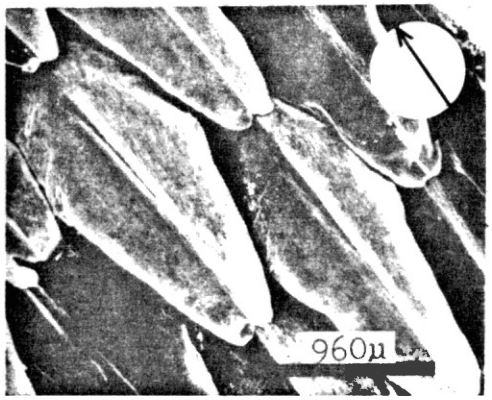
b.)



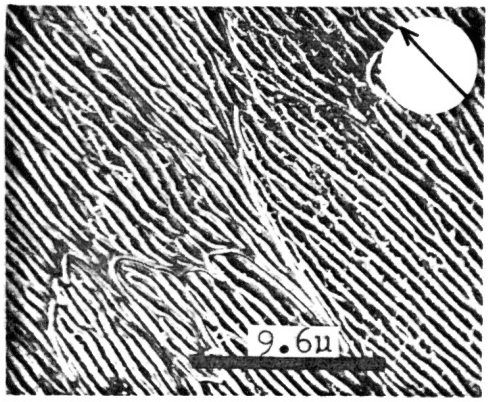
c.)



d.)



e.)



Only specimen OSUR1313 clearly shows a surface pattern at higher magnification. The pattern consists of numerous small ridges that anastomose slightly. Larger longitudinal ridges (character 4) are formed as superimposed folds on the scale surface. The scale surface seems to be composed of irregular, compartment-like,units bearing the small ridges. Compartment borders are partially outlined by a smalll terminal ridge posteriorly. The numberous small ridges simply stop at this border. Each compartment fits against its neighbor tightly (Figure 21).

Thamnophis cyrtopsis. Scales of Thamnophis cyrtopsis resemble those of T. butleri at low magnification. At moderate magnification (290X) the longitudinal ridges are prominant but the detail of the surface is obscured.

Thamnophis elegans. Dorsal midbody scales of Thamnophis elegans are, at low magnification, quite similar to those of T. butleri. The longitudinal ridges are somewhat more prominent, however, and at moderate magnifications stand out in sharp relief against the inter-ridge surfaces. The inter-ridge trough areas appear to be crossed by a network of veins or irregular cross bridges. At higher magnification the scale surface consists of numerous individual compartments, each of which is bounded by a ridge that is curved to form an irregular polygonal outline. The surface of each polygonal unit has numerous longitudinal ridges that anastomose slightly. The longitudinal ridges visible at low

magnification consist of many short ridges apparently derived from the surface ridge pattern. Some of the short ridges emerge from the surrounding areas and join the longitudinal ridges in distinct chevron-like patterns (Figure 22).

Of the four specimens studied, only one presents a clear, sharp surface pattern when viewed at high magnification (OSUR1450), the others show varying amounts of surface obstruction.

Thamnophis eques. At low magnification scales of Thamnophis eques are similar to those of other Nerodia and Thamnophis described earlier. No apical pits are visible, but a central keel and longitudinal ridges are readily discernible (Figure 22). Higher magnification shows a scale surface consisting of closely spaced small ridges all running essentially parallel to the scale axis. The larger longitudinal ridges visible at low magnification consist of an uplifted group of several small ridges tightly packed together (Figure 22).

Thamnophis marcianus. Dorsal midbody scales of Thamnophis marcianus are similar to other Thamnophis at low magnification. At higher magnification a variation in surface appearance is evident. Specimen OSRU1494 shows almost no surface structure; the scales appear to be completely coated with a thick film. Specimen OSUR2117 possesses a similar surface coating, but the coating is

Figure 22. *Thamnophis* Scales

a.) *T. elegans* High Magnification Surface Pattern

b.) *T. eques* Moderate Magnification Overview

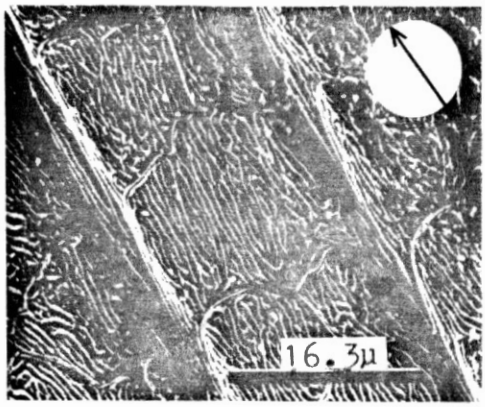
c.) *T. eques* High Magnification Surface Pattern

d.) *T. marcianus* High Magnification Scale Surface

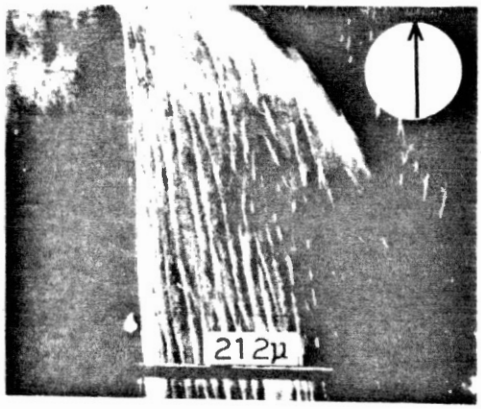
e.) *T. marcianus* High Magnification Shed Surface

f.) *T. proximus* High Magnification Surface Pattern

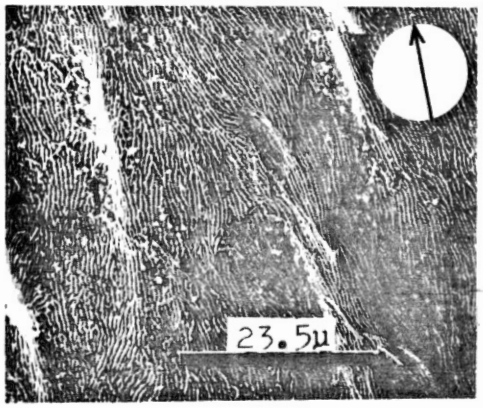
a.)



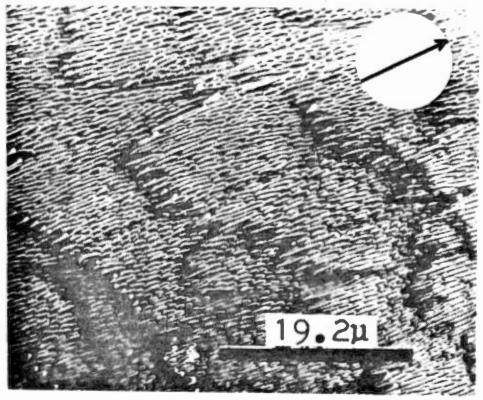
b.)



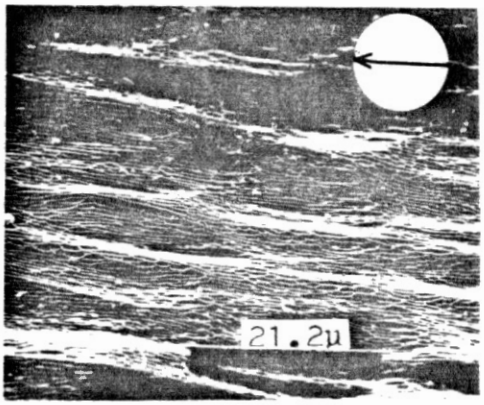
c.)



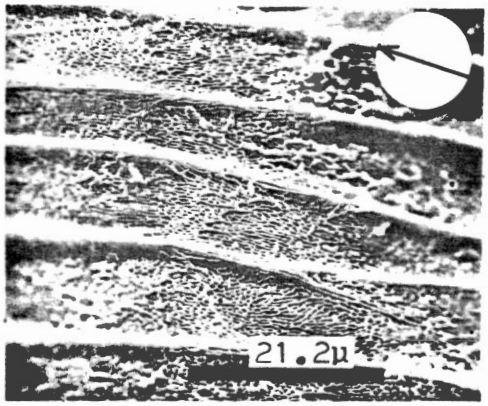
d.)



e.)



f.)



interrupted at various places allowing patches of surface pattern to be seen. The remaining specimens all show a surface pattern of some form, although OSUR1241 shows a flattened and dirty pattern compared to OSUR3884 and OSUR-3885, both of which show clearer and more distinct surface sculpturing. A comparison was made between fresh scale surfaces and shed patterns of OSUR3885. The shed was carefully removed with fine forceps, washed, dried, and coated just like any other specimen. Concurrently, a portion of the newly exposed surface was also processed. Observation of these two surfaces at 2200X shows essentially similar patterns on both preparations (Figure 22). The shed appears much more delicate and seems to have been somewhat deformed during processing.

At high magnification the surface consists of anastomosing ridges with relatively little space between adjacent ridges. Longitudinal ridges, conspicuous at low magnification, are less conspicuous at higher magnification and appear to be little more than folds in the scale surface.

Thamnophis proximus. At low magnification dorsal mid-body scales of Thamnophis proximus are typical of the genus. Of the two specimens studied only one shows detailed surface structure at increased magnification (OSUR3663). The surface is marked by longitudinal ridges with lattice-work in between. At high magnification the surface is covered by numerous, small anastomosing ridges. The longitudinal

ridges result from periodic foldings in the surface and show no special substructure (Figure 22).

Thamnophis radix. Low magnification of dorsal midbody scales of Thamnophis radix presents a typical Thamnophis appearance; i.e., elongate scales, prominent central keel, longitudinal striations, and no pits. Specimen OSUR531 shows no detailed pattern at higher magnification. Specimen OSUR598 shows a sharply detailed pattern on certain scale surfaces, but this depends upon area examined (Figure 23). Some scales have been shed but other "old" scales remain loosely attached to the underlying tissue. When viewed simultaneously, the shed or top pieces show sharper, detailed surface structures, whereas the lower "new" surface looks shrivelled and heavily coated by a film (c.f. Nerodia sipedon)(Figure 6).

High magnification of the "good" surfaces shows a pattern similar to that of Thamnophis elegans. Longitudinal ridges project from the surface with rather broad shallow troughs between. Troughs appear to be filled by compartment-like units which give rise to chevron processes. These processes project posteriorly in linear array to form the longitudinal ridges (Figure 23).

Thamnophis sauritus. Low magnification of dorsal midbody scales of Thamnophis sauritus reveals a morphology similar to other Thamnophis species. Only specimen OSUR-2738 shows surface detail at higher magnification. The

Figure 23. *Thamnophis* Scales

a.) *T. radix* Moderate Magnification Surface Pattern

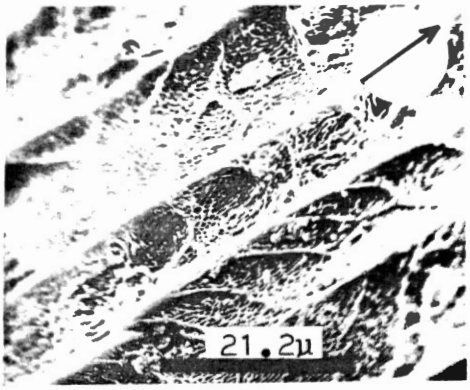
b.) *T. radix* High Magnification Surface Pattern

c.) *T. sauritus* High Magnification Surface Pattern

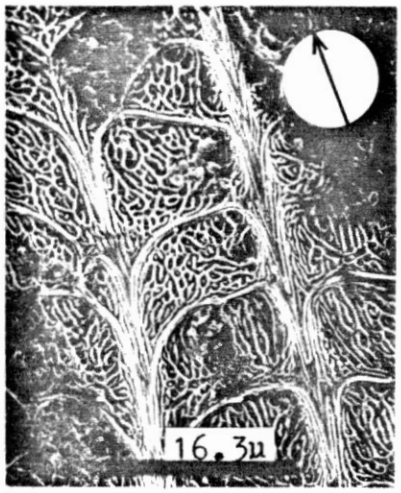
d.) *T. sirtalis* Moderate Magnification Surface Pattern

e.) *T. sirtalis* High Magnification Surface Pattern

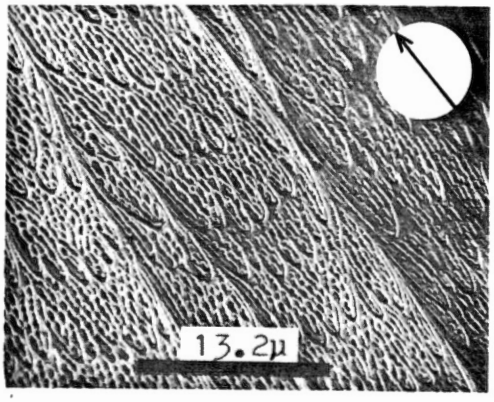
a.)



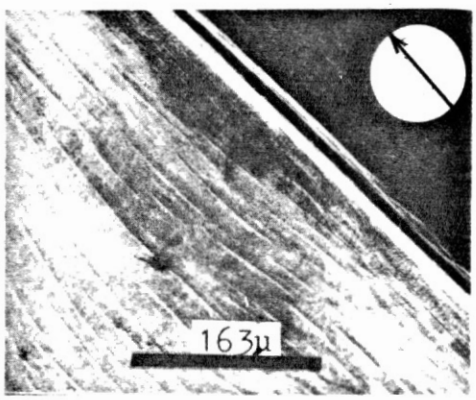
b.)



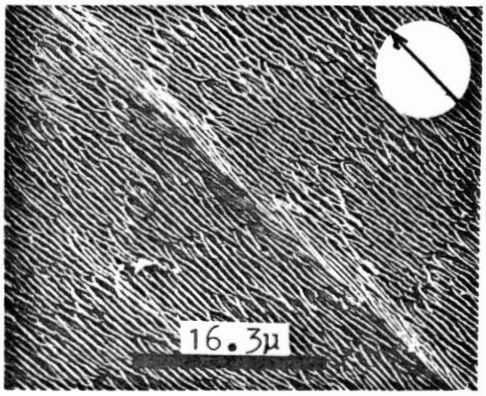
c.)



d.)



e.)



pattern consists of polygonal compartment-like units whose surfaces are covered by anastomosing ridges. Each unit is bordered by a ridge which is scalloped posteriorly. Adjacent units are tightly spaced. Longitudinal ridges are upfoldings formed by overlapping chevron-like extensions from adjacent units (Figure 23).

Thamnophis sirtalis. Dorsal midbody scales of Thamnophis sirtalis resemble those of other Thamnophis at low magnifications (Figure 23). High magnification shows that longitudinal ridges seem to arise and disappear randomly from the scale surface. Individual unit structures are less distinct than those on T. sauritus scales, but present. The surface of each unit is covered by small essentially parallel ridges (ridges do not anastomose) (Figure 23).

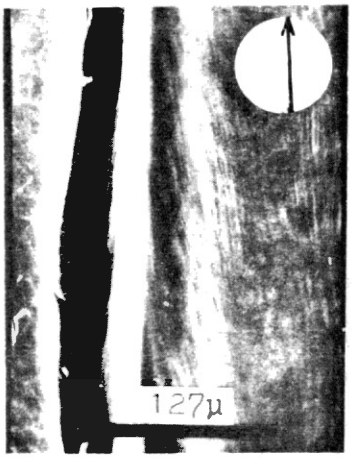
Tropidoclonion lineatum. Dorsal midbody scales of Tropidoclonion lineatum show similar morphology to those of Nerodia and Thamnophis at low magnification; as in Thamnophis no pits are visible (Figure 24). Specimen OSUR2285 shows no surface detail. Specimen OSUR1107 resembles Nerodia sipedon OSUR491 in presenting a partially exposed new scale surface. Both old and new scale surfaces may be observed simultaneously (Figure 24). The old outer surface is devoid of surface pattern, whereas the new surface shows a pattern of longitudinal ridges or striations at moderate magnification. Higher magnification shows a pattern of anastomosing ridges on a folded scale surface. The folds

Figure 24. Tropidoclonion Scales

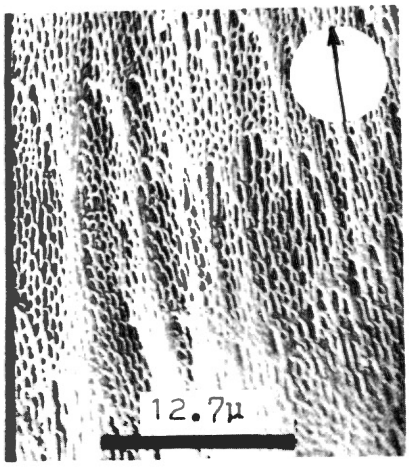
a.) Moderate Magnification Surface Pattern (The bright region running vertically through the phtotgraph is the central keel. The smooth bright surface to the left of the keel is part of the old outer surface-- to the right is the new sharply sculptured surface.)

b.) High Magnification Surface Pattern

a.)



b.)



give rise to the longitudinal ridges visible at lower magnifications (Figure 24). The pattern resembles that of Thamnophis marcianus shed.

CHAPTER IV

DISCUSSION

Eyes

A summary of visual cell types present in the retinae of the 9 species studied is presented in Table V. Natricine species are similar to the typical diurnal pattern described by Walls (1942); i.e., Types A, B, and C cones. Two exceptions were noted however. First, Nerodia rhombifera had an additional cell type probably corresponding to Walls' Type C' (or Type D of Underwood). Secondly, the Type C cells in the retina of Storeria dekayi are very rod-like in morphology. This may represent adaptation to a secretive activity preference. Walls (1942) stated that other secretive, crepuscular, or nocturnal colubrids show similar modification of the Type C cells (e.g., Arizona, Cemophora, or Lampropeltus). It would be useful to study other secretive natricine species such as Regina alleni or Virginia striatula for comparison of Their Type C cones to Storeria.

Three non-natricine species studied (i.e., Agkistrodon contortrix, A. piscivorus, and Farancia abacura) conform to published accounts of their retinal cell types (Walls, 1942; Underwood, 1970). The visual cell types present in these species were thought to represent adaptation to nocturnality

TABLE V

VISUAL CELL TYPES IDENTIFIED

Species	Cones	Rods
Agkistrodon contortrix	A,B	C
Agkistrodon piscivorus	A,B	C
Farancia abacura	A,B,C	C' (D)
Nerodia erythrogaster	A,B,C	
Nerodia fasciata	A,B,C	
Nerodia rhombifera	A,B,C	C' (D)
Pelamis platurus	A,B,C	
Regina grahami	A,B,C	
Storeria dekayi	A,B,C*	
Thamnophis proximus	A,B,C	

* Type C cones of *Storeria* are very elongate and slender.

by Walls.

The remaining species, Pelamis platurus, has a pure-cone, diurnal type retina (Walls, 1942). Neither Walls nor Underwood (1967a, 1970) studied Pelamis but suggested that some elapids do have a Type A, B, and C cone retinal pattern, as reported here. Hibbard and Lavergne (1972) failed to identify any Type B cells in their study of Pelamis, but they were present in the specimen I studied.

In addition to data on visual cell types, data were also collected on several numerical features of the retina. Parameters chosen for study are relatively easy to obtain in a reproducible manner from a variety of specimens and preparations. Nuclei stain well and are prominent structures in retinal sections. Nuclear counts are straightforward and may be used to estimate the proportion of each cell type present in the retina; e.g., visual cells, interneurons, etc. The relative number of cell types in the retina has been used in previous discussions of the phenomena of convergence or divergence of visual information in retinae and in various comparative discussions of snake eyes (Walls, 1942; Underwood, 1970; Stovall, 1975, 1976b).

Overall similarity of the retinae studied was inferred from correlation analysis of receptor vs. interneurons and receptors vs. ganglion cells. Analyses were performed using 3 species groups; i.e., natricine species only, all species studied, and colubrid species only (Table VI). These analyses show significant correlation among natricine and

TABLE VI

CORRELATION ANALYSIS OF RETINAL COUNTS

Character Comparison	Natricinae				Colubridae				All Snakes			
	N	df	r	p	N	df	r	p	N	df	r	p
receptor cells - interneurons	7	5	.897	< .05*	8	6	.789	< .05*	11	9	.08	> .10
receptor cells - ganglion cells	7	5	.958	< .05*	8	6	.878	< .05*	11	9	.186	> .10

* significant at 0.05 level

colubrid species. Neither receptor vs. interneuron nor receptor vs. ganglion cell relationships are correlated among all species.

The correlation analysis indicates that these retinal features are not highly variable within the subfamily--these characters are probably conservative evolutionarily and are related to phyletic relationships of the group. For similar reasons, Walls (1942) and Underwood (1967a, 1970) used retinal patterns in the construction of phylogenies of snake visual cells and snakes in general.

The relative number of receptor cells compared to interneuron cells can be used to make functional inferences about the retina. Both species of Agkistrodon have roughly equal numbers of receptors and interneurons. Farancia and Pelamis have almost 3 times more interneurons than receptor cells. In natricine species interneurons outnumber receptor cells some 4-6 times (Appendix D). Interneurons consist of three types: horizontal, amacrine, and bipolar cells. Bipolar cells transmit impulses and hence visual information from receptors to ganglion cells. The other two classes of interneurons are involved in lateral information processing (Rodieck, 1973). The greater the number of interneurons the greater the capacity of intraretinal processing. Further, the higher the number of bipolar cells the more representation each receptor has at the ganglion cell level. In general diurnal, pure-cone retinae have a high number of interneurons compared to receptor cells (Walls, 1942).

Nocturnal, rod-dominant retinae usually have relatively fewer interneurons. Walls (1942) and others argued this arrangement results in greater acuity in pure-cone retinae compared to rod-dominant retinae. If so, Agkistrodon retinae are less acute than the natricine species or Farancia or Pelamis since Agkistrodon interneurons are 1/3 to 1/6 times less numerous (relative to the receptors).

Since information leaves the retina via the ganglion cells, it is also important to consider the relative number of receptors to ganglion cells. Agkistrodon species average ca. 8.5 times more receptors than ganglion cells, whereas the natricine species studied avarage only about 1.5 times more receptors than ganglion cells. As with the interneuron numbers, both Farancia and Pelamis have lower ganglion cell numbers than any of the natricine snakes (about 2.25 times more receptors than ganglion cells). Since visual information from receptors and bipolars converges onto ganglion cells, a high ganglion cell number (relative to receptors) implies relatively less convergence and thus more direct representation for each receptor in the brain (Walls, 1942). Again, rod-dominant retinae like those in Agkistrodon are less acute (i.e., the information output to the brain is more generalized) than pure-cone retinae such as found in the other species studied.

Hibbard and Lavergne (1972) reported observations of feeding behavior of Pelamis platurus. They inferred from the random, thrashing behavior that Pelamis may have rather

poor (non-acute) vision. They pointed out, however, that in the absence of appropriate physiological tests such assessment is highly speculative. Based on present data, Pelamis is likely to have reasonably acute vision, at least compared to Agkistrodon. Any potential or actual lack of visual sensitivity in crotalids is compensated for by a highly sensitive heat receptor organ (Bullock and Barrett, 1968; Meszler, 1970).

Habitat preferences among all the snakes studied range from aquatic to semi-aquatic to terrestrial (see Introduction). The fully aquatic Pelamis possesses a retina similar in visual cell types to the natricine species in the study, both semi-aquatic and terrestrial. Further, retinae of both the crotalids are similar to each other despite differences in affinity for water. Within the natricine species studied habitat preference varies from semi-aquatic to terrestrial, although there is overlap. The semi-aquatic species (Nerodia erythrogaster, N. fasciata, N. rhombifera, Regina grahami) were pooled and compared to the more terrestrial species (Storeria dekayi pooled with Thamnophis proximus) via two-sample t-tests. Percentages of receptor cells, interneurons, ganglion cells, Type A cells, Type B cells, and Type C cells were compared. No significant differences were detected among any of these categories (Table VII). Thus affinity for water or aquatic habits is not reflected in retinal structure.

To facilitate analysis, Nerodia erythrogaster, N.

TABLE VII

COMPARISON OF RETINAL PARAMETERS BETWEEN SEMI-AQUATIC AND TERRESTRIAL NATRICINAE[1]

Character	Semi-aquatic			Terrestrial					
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	P	df
receptor cells	14.18%	1.27%	5	12.2%	.14%	2	2.13	> .05	5
interneruons	76.62%	1.65%	5	79.50%	1.13%	2	2.26	> .05	5
ganglion cells	9.18%	.76%	5	8.35%	1.34%	2	1.10	> .10	5
Type A cells	72.94%	10.10%	5	87.05%	8.13%	2	1.74	> .10	5
Type B cells	13.76%	8.57%	5	8.10%	2.55%	2	0.73	> .10	5
Type C cells	11.98%	4.93%	5	4.85%	5.59%	2	1.92	> .10	5

[1]percents were transformed by arc sine before statistical analysis

fasciata, N. rhombifera, Regina grahami, and Thamnophis proximus were pooled and designated variable in activity as opposed to secretive habits of Storeria dekayi. One sample t-tests were applied to the retinal parameters (Table VIII). Significant differences were present only in percentage of Type C cells (0.05 level). Examination of the numerical data (Appendix D) for Storeria suggests that the percentage of Type C cells is likely to be unreliable, an implication that is supported from review of the slide material. Due to extremely small eye size (2-3 mm in diameter) and accompanying tissue processing difficulties cell type diagnosis is very difficult.

Activity preferences are mostly diurnal for the species considered here, however certain species are preferentially nocturnal (see Introduction). The crotalid species have what Walls (1942) described as a nocturnally adapted eye, with an elliptical pupil and rod-dominant retina. The remaining species have diurnal-type eyes (i.e., round pupil and pure-cone or cone-dominant retinae). Most Nerodia species have been observed to be nocturnally active in hot weather (Conant, 1975), and except for N. rhombifera they possess pure-cone retinae. Thus these snakes remain diurnal in overall retinal structure even though they may be night active.

Underwood (1970) stated that Vipera berus was the only snake that had been studied in any detail by electron microscopy although he provided some ultrastructural data on

TABLE VIII

COMPARISON OF RETINAL PARAMETERS BETWEEN VARIABLY ACTIVE AND SECRETIVE NATRICINAE[1]

Character	Variably Active			Secretive			
	$\bar{X}$	SD	N	μ	t	P	df
receptor cells	13.87%	.95%	6	12.10%	1.22	> .10	5
interneurons	77.23%	2.11%	6	78.70%	.63	> .10	5
ganglion cells	8.88%	1.00%	6	9.30%	.40	> .10	5
Type A cells	74.33%	9.65%	6	92.80%	2.00	> .10	5
Type B cells	13.12%	7.82%	6	6.30%	.77	> .10	5
Type C cells	11.45%	4.59%	6	.90%	3.26	< .05*	5

[1]percents were transformed by arc sine before statistical analysis
*significant at 0.05 level

Heterodon platyrhinos as well as Vipera. Since 1970 electron microscope studies have been reported on Coluber constrictor, Hypsiglena torquata (Stovall, 1975, 1976a,b), Leptodeira annulata (Stovall, 1975; Miller and Snyder, 1977), Pelamis platurus (Hibbard and Lavergne, 1972), and Hydrophis melanocephalus (Hibbard, 1975). From a review of these data, it is evident that retinal ultrastructure, on the whole, is similar among snakes studied.

Electron microscope observation shows a variety of intra-ellipsoid inclusions. In Vipera, Bothrops, and Coluber there are electron dense granules within ellipsoid mitochondria. In Hypsiglena and Thamnophis ellipsoids, peripheral mitochondria contain vesicles of varying electron densities. The central region of both Hypsiglena and Thamnophis ellipsoids lacks well defined mitochondria but instead is filled with electron lucid vesicles and vesicles of varying electron opacity (Thamnophis).

The electron dense granules found in ellipsoid mitochondria of all species studied, except Pelamis, conform in structure to those described by Yamada, Ishikawa, and Hatae (1966) in Elaphe climacophora. The granules in Elaphe were demonstrated to be Sudan Black B positive, indicating a lipid constituency. This constrasts with the description of electron dense granules in mitochondria of the Pelamis ellipsoid. Hibbard and Lavergne (1972) argued that these granules in Pelamis differ from those in other species as described in the literature in resembling glycogen aggre-

gates and in being PAS positive, a characteristic of glycogen.

The role of these vesicles or granules is not certain. It has been established, however, that the inner segments of visual cells serve a wave-guide function to channel light onto the outer segments (Tansley and Johnson, 1956; Yamada et al., 1966; Miller and Snyder, 1977; and others). It is likely then that these vesicles or granules, either wholly intramitochondrial or mitochondria-derived, replace the oil droplets and paraboloids present in the inner segment of other vertebrates as Underwood (1970) suggested in his descriptions of the "refringent bodies" he observed in snake ellipsoids in light microscope preparations.

The oil droplet-like structure seen in Thamnophis sections is of interest, particularly in view of Underwood's (1970) statement that snake visual cells lack oil droplets and paraboloids. In micrographs of oil droplets in other vertebrates, there does not seem to be a dense marginal zone surrounding oil droplets (Pedler and Tansley, 1963; Young, 1977). The paraboloids described by Pedler and Tansley (1963) did show a dense marginal layer, however. Such a dense marginal layer is present in the structures I observed in Thamnophis. Paraboloids are found at the proximal end of the ellipsoids, however, and not at the distal end as are oil droplets (Walls, 1942; and others) and as are the structures observed in Thamnophis.

The possibility that the droplet-like structures

represent artifact is supported by the fact that the structures are scattered sparsely and not found in every specimen. Oil droplets seem to be uniformly distributed when present in the retinae of a diurnal lizard, turtle, or bird (Walls, 1942; Underwood, 1970; and others).

If these structures are oil droplets, they must represent independently evolved structures in this species (or at least within a small group) since they have not been reported in any other snakes. The number of individuals studied in the genus Thamnophis is small, however, and a broader survey is needed to confirm the presence of such anomalous structures.

The finding of phagosomes in Storeria dekayi pigment epithelium emphasizes the fundamental similarity among vertebrate retinae. Earlier failures to find such inclusions in pigment epithelium of snakes is probably due to time of day. The timing of shedding outer segment lamellae depends on whether or not one considers rods or cones. The low-light receptors (rods) shed and thus produce phagosomes shortly after dawn. This accounts for finding phagosomes in rod-dominant retinae processed in the daytime (the typical case). Cones do not shed outer segments and hence do not produce phagosomes in the daytime, and therefore retinae processed in the daytime typically do not show cone-produced phagosomes (see Young, 1977). Cones do shed outer segment lamellae but only after onset of darkness. This means that retinae should be processed at night to demonstrate cone-

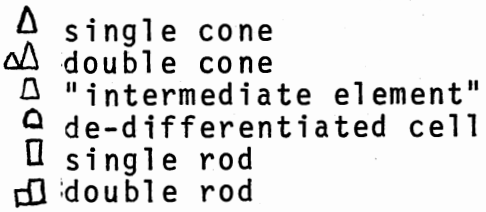
produced phagosomes optimally. A further complication is that prolonged periods of continuous light may disrupt or stop shedding altogether (Hollyfield and Basinger, 1978).

Walls (1942) surveyed the eyes and retinae of many species of reptiles and other vertebrates and compiled the most comprehensive analysis of the evolutionary history of vertebrate ocular anatomy yet published. Based on his studies, Walls developed a scheme of evolutionary development of the vertebrate eye, including that of snakes. In his scheme, Walls presented a logical basis for the origin of snakes from burrowing lizard ancestors (an origin supported on other data as well, see Bellairs and Underwood, 1951).

The only worker to significantly enlarge and improve on Walls' work is Underwood. Underwood's work on reptilian retinae culminated in A Contribution to the Classification of Snakes (1967). Underwood reviewed snake retinal patterns and suggested a phylogeny in subsequent works, notably in The Eye, Biology of the Reptilia (1970).

Since the work of Walls and Underwood is so important, a brief review of their phylogenies is appropriate (Figures 25 and 26). Walls and Underwood agreed that ancestral snakes had degenerative retinae and therefore very simple visual cells. The retina of Typhlops possesses a single rod-like type of visual cell (Scolecophidian pattern in Underwood's scheme). The Typhlops type retina may be similar to the ancestral form or may represent a secondary

Figure 25. Walls' Phylogeny of Snake Visual Cells



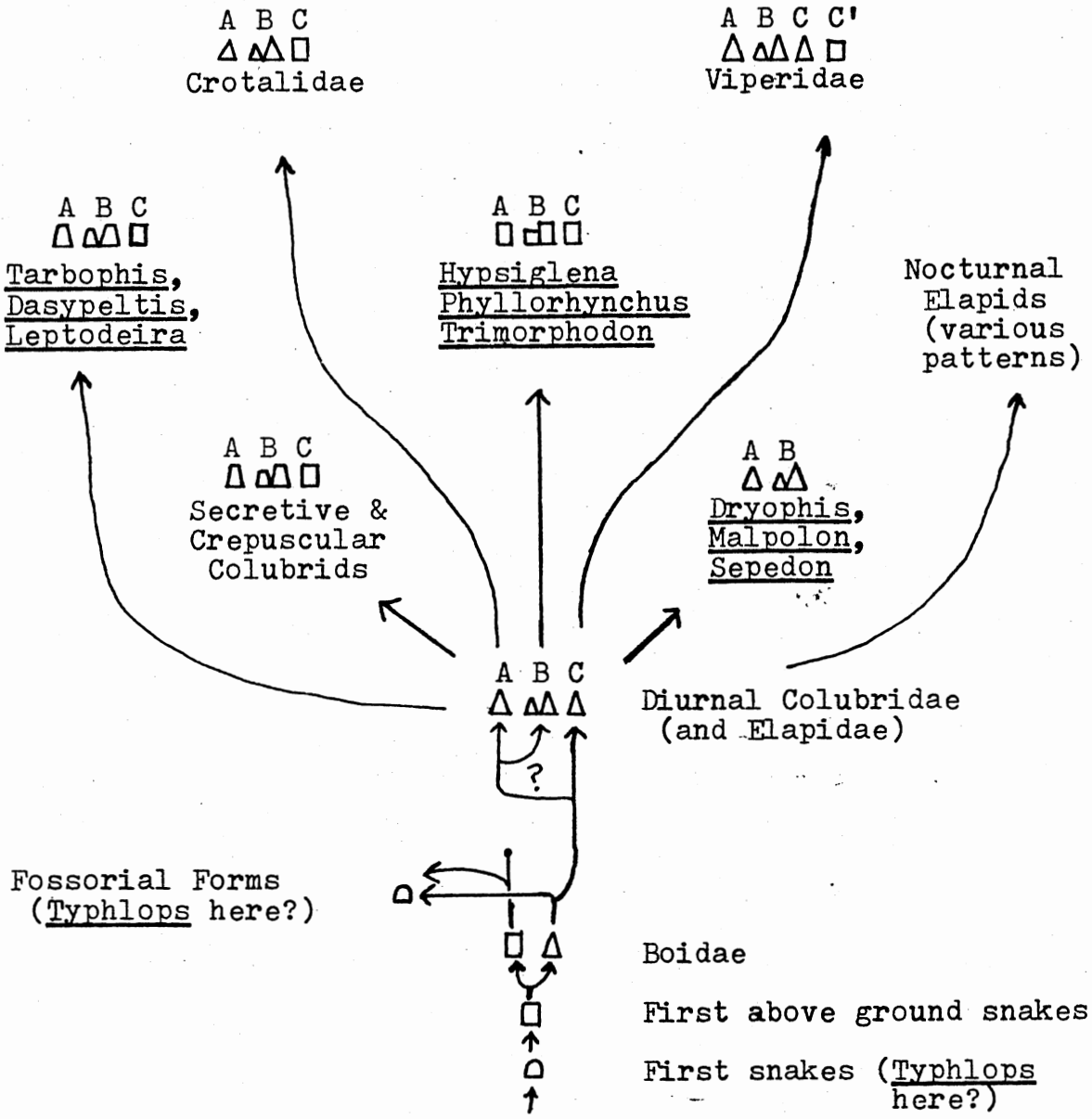
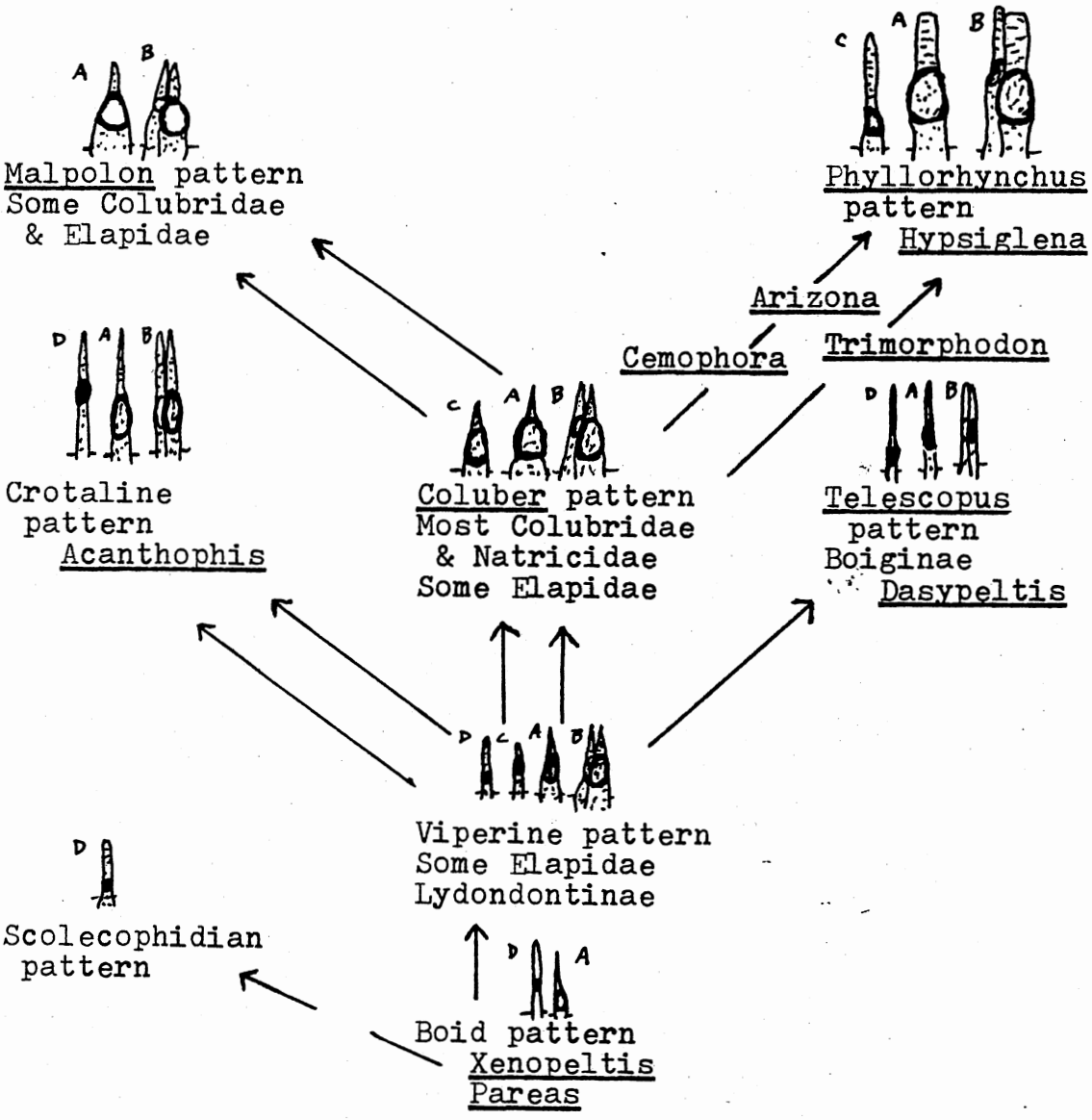

A B C
Crotalidae
A B C C'
Viperidae
A B C
Tarbophis,
Dasypeltis,
Leptodeira
A B C
Hypsiglena
Phyllorhynchus
Trimorphodon
Nocturnal
Elapids
(various
patterns)
A B C
Secretive &
Crepuscular
Colubrids
A B
Dryophis,
Malpolon,
Sepedon
A B C
Diurnal Colubridae
(and Elapidae)
?
Fossorial Forms
(Typhlops here?)
Boidae
First above ground snakes
First snakes (Typhlops
here?)

Figure 26. Underwood's Phylogeny of Snake Visual Cells


A
B
Malpolon pattern
Some Colubridae
& Elapidae
C
A
B
Phyllorhynchus
pattern
Hypsiglena
Arizona
Cemophora
Trimorphodon
D
A
B
Crotaline
pattern
Acanthophis
C
A
B
Coluber pattern
Most Colubridae
& Natricidae
Some Elapidae
D
A
B
Telescopus
pattern
Boiginae
Dasypeltis
D
C
A
B
Viperine pattern
Some Elapidae
Lydondontinae
D
Scolecophidian
pattern
D
A
Boid pattern
Xenopeltis
Pareas

degeneration (as it must in Underwood's scheme). Boids were believed to be the most primitive snakes on the basis of various data and the simple duplex boid retina composed of one rod-type and one cone-type element is thought to be the starting place from which other, more modern groups diverge. Walls and Underwood disagreed on the next step, however. Walls suggested that the diurnal pattern represented by Coluber, Nerodia, etc. evolved directly from the boid pattern. Most snakes studied show this pattern and it was not illogical for Walls to make this conclusion. Walls saw all other patterns as specializations for different adaptive habits assumed by snakes that deviated from the strictly diurnal mode.

In contrast, Underwood viewed the viperine retina as directly derived from boids. Further, the viperine pattern was viewed as ancestral to all other groups. Underwood, using a broader data base, argued that the viperine pattern is widely distributed in a variety of higher snakes including Micrurus, Farancia, Abastor, Heterodon, Coronella, as well as viperids. Underwood (1967:53) stated: "In the diurnal Natricidae and Colubridae there are types A and B cones and small C cones (Dromicus, Natrix, Thamnophis, Coluber, Drymarchon) the type D element has evidently been lost altogether (save Coronella)."

The presence of type D cells in Nerodia rhombifera may be explained in both Walls and Underwood terms. If the pure cone Type A, B, and C pattern is ancestral, as Walls sug-

gested, the Type D cells in N. rhombifera represent a new development, perhaps related to summertime nocturnality. If, however, the viperine pattern is ancestral, then Type D cells in N. rhombifera represent a retension of the primative condition. As Underwood noted, Type D cells are sporadically distributed among colubrid snakes. Walls' interpretation thus requires a series of independent Type D development events compared to a single development and subsequent degeneration in Underwood's scheme.

Scales

Dendrograms produced by single, complete, and average linkage clustering are shown in Figures 27, 28, and 29. The dendrograms are somewhat different from those typically found in the literature due to the low number of characters (8) used in the species-by-species comparison. The low character number results in several species pairs showing a 1-to-1 correspondence; i.e., linked at the 1.0 level. Aside from this feature, however, these dendrograms are typical in appearance.

Single linkage clustering links all 19 species at no lower than the 0.75 level, showing a high degree of similarity among the 19 species, whereas complete linkage, the more conservative procedure, links all 19 species at the 0.38 level. Average linkage links all 19 species at the 0.48 level.

All three clustering methods show a high degree of

Figure 27. Single Linkage Dendrogram

Species names are abbreviated as follows:

1	Agk pis	*Agkistrodon piscivorus*
2	Far aba	*Farancia abacura*
3	Ner fas	*Nerodia fasciata*
4	Ner rho	*Nerodia rhombifera*
5	Ner sip	*Nerodia sipedon*
6	Reg all	*Regina alleni*
7	Reg rig	*Regina rigida*
8	Reg sep	*Regina septemvittata*
9	Sto dek	*Storeria dekayi*
10	Sto occ	*Storeria occipitomaculata*
11	Tha but	*Thamnophis butleri*
12	Tha ele	*Thamnophis elegans*
13	Tha equ	*Thamnophis eques*
14	Tha mar	*Thamnophis marcianus*
15	Tha pro	*Thamnophis proximus*
16	Tha rad	*Thamnophis radix*
17	Tha sau	*Thamnophis sauritus*
18	Tha sir	*Thamnophis sirtalis*
19	Tro lin	*Tropidoclonion lineatum*

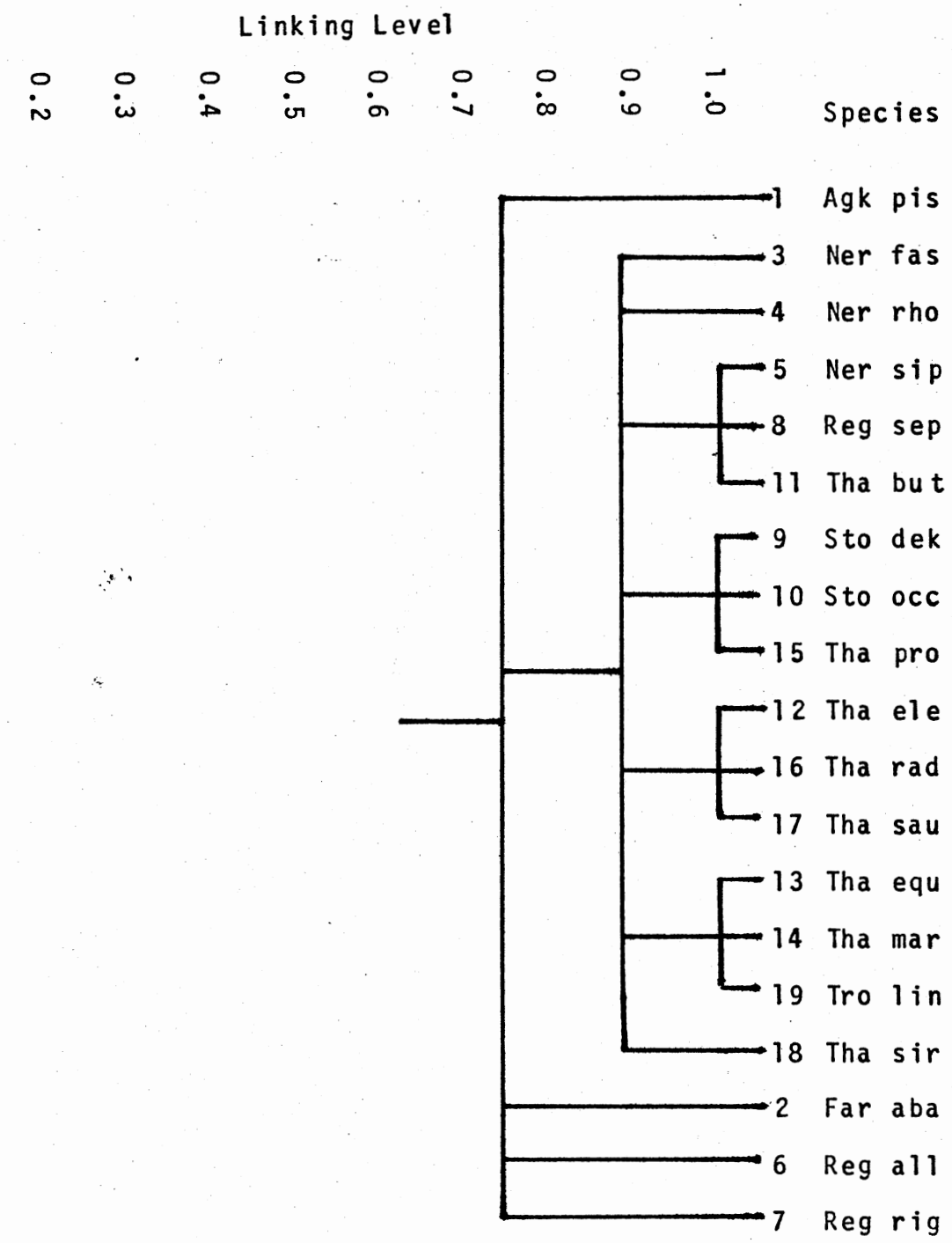

Linking Level
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Species
1 Agk pis
3 Ner fas
4 Ner rho
5 Ner sip
8 Reg sep
11 Tha but
9 Sto dek
10 Sto occ
15 Tha pro
12 Tha ele
16 Tha rad
17 Tha sau
13 Tha equ
14 Tha mar
19 Tro lin
18 Tha sir
2 Far aba
6 Reg all
7 Reg rig

Figure 28. Average Linkage Dendrogram

Species abbreviations given in legend to Figure 27

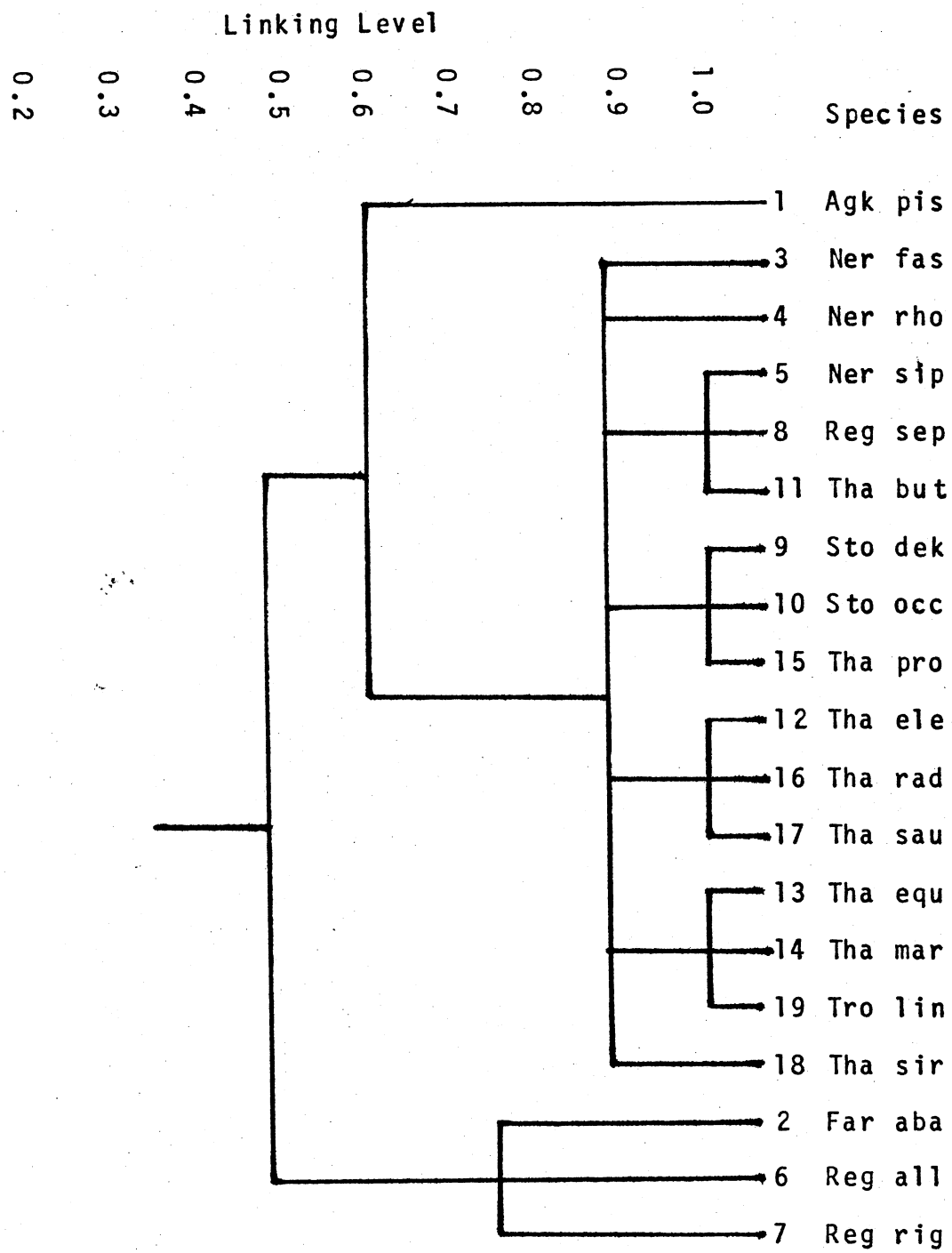
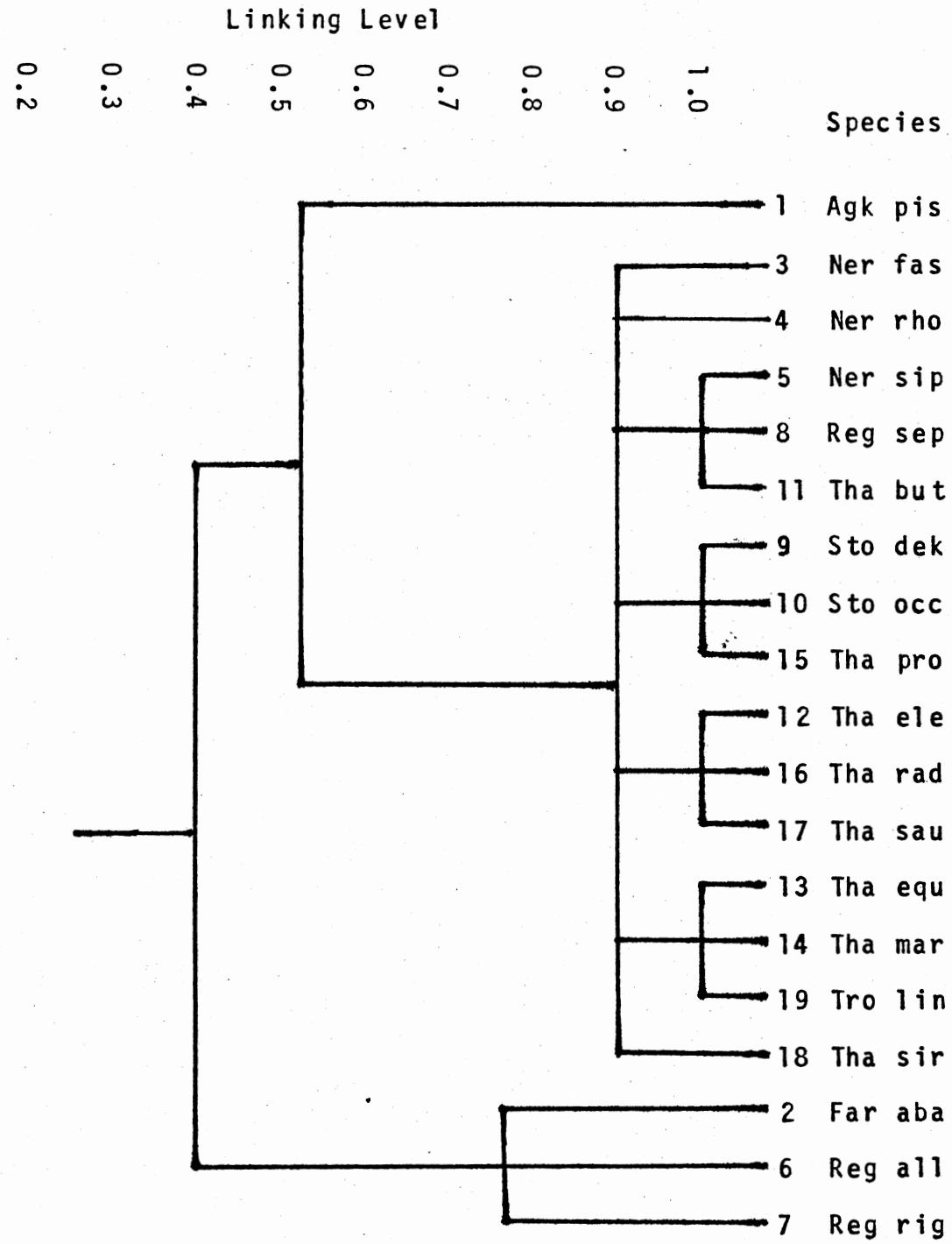

Linking Level
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Species
1 Agk pis
3 Ner fas
4 Ner rho
5 Ner sip
8 Reg sep
11 Tha but
9 Sto dek
10 Sto occ
15 Tha pro
12 Tha ele
16 Tha rad
17 Tha sau
13 Tha equ
14 Tha mar
19 Tro lin
18 Tha sir
2 Far aba
6 Reg all
7 Reg rig

Figure 29. Complete Linkage Dendrogram

Species abbreviations given in legend to Figure 27


Linking Level
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Species
1 Agk pis
3 Ner fas
4 Ner rho
5 Ner sip
8 Reg sep
11 Tha but
9 Sto dek
10 Sto occ
15 Tha pro
12 Tha ele
16 Tha rad
17 Tha sau
13 Tha equ
14 Tha mar
19 Tro lin
18 Tha sir
2 Far aba
6 Reg all
7 Reg rig

similarity (1.0 level) among four groups of snakes: (1) Nerodia sipedon, Regina septemvittata, Thamnophis butleri; (2) Storeria dekayi, S. occipitomaculata, Thamnophis proximus; (3) Thamnophis elegans, T. radix, T. sauritus; (4) Thamnophis eques, T. marcianus, Tropidoclonion lineatum. All three techniques also link Nerodia fasciata, N. rhombifera, and Thamnophis sirtalis with these four groups at the 0.88 level. All three techniques link Farancia abacura, Regina alleni, and R. rigida at the 0.75 level, but diverge in the linkage of remaining species.

The average linkage dendrogram is discussed below, but the reader can easily compare the single and complete linkage dendrograms by reference to Figures 27, 28, and 29. Below the 0.75 level, Agkistrodon piscivorus is grouped with all the Nerodia and Thamnophis species at the 0.61 level. Finally, Agkistrodon, Nerodia, and Thamnophis species are linked with Farancia, Regina alleni, and R. rigida at the 0.48 level.

Ecologies of the species studied are more diverse than the scale patterns. Species of Nerodia and Thamnophis show an overall similarity in scale patterns even though ecologies range from semi-aquatic to terrestrial. This correlates with biochemical studies (George and Dessauer, 1970) that show Nerodia to be more similar to Thamnophis than to Old World species formerly included with Nerodia in Natrix. Tropidoclonion lineatum likewise shows a high level of similarity to Nerodia and Thamnophis, which is consistent

with Conant's (1975) statement of ecological and morphological similarity between Thamnophis and Tropidoclonion.

Regina contains four species, three of which are included in the cluster analyses. R. grahami was included in the study as well, but poor specimen quality prevented determination of all character states utilized in the analyses. This group of crayfish-eating snakes probably diverged from a Nerodia-like ancestor with grahami representing the most primitive and alleni the most advanced of the group (Rossman, 1963). R. septemvittata is more similar to Thamnophis than to either of the other Regina. Scales of R. rigida and R. alleni are increasingly more dissimilar to Thamnophis, however. Good specimens of R. grahami would be expected to be similar to Thamnophis, as is R. septemvittata, if Rossman was correct in his phylogeny of the genus.

Regina alleni and R. rigida are more similar to Farancia abacura than to the other snakes studied. This may be explained on the basis of convergent adaptation. Gans and Baic (1977) related a smooth, glossy scale surface to fossorial habits and Farancia and R. alleni are the most fossorial snakes in the study. Adaptation to fossorial habits may also be related to loss of central keels from the scales (Jackson and Reno, 1975). Farancia and R. alleni are the only colubrid snakes studied here that lack central keels. In support of a close relationship among alleni and other Regina, however, Rossman (1963) reported that some

scales are keeled in the tail and supra-anal region of R. alleni. Monroe and Monroe (1968) reported a study of scale surfaces of Drymarchon corais. Drymarchon has iridescent scales (as does Farancia) and is semi-fossorial in habits (it is sometimes commonly called a gopher snake in Florida because of its occurrence in gopher tortoise burrows). Electron micrographs of Drymarchon scales revealed a pattern similar to Farancia. Monroe and Monroe (1968) found the pattern to produce a diffraction grating, thus explaining the iridescence. Iridescence would seem to be a by-product of a pattern that reduces adhesion of soil particles and thus friction (Gans and Baic, 1977).

Paired apical or scale pits located near the posterior end of the scales were observed on only five of the twenty-five species studied (Agkistrodon contortrix, A. piscivorus, Nerodia cyclopion, N. fasciata, N. rhombifera). Cope (1900) used the presence of apical pits to distinguish Natrix from Thamnophis. The presence of apical pits in Natrix and thier absence in Thamnophis was utilized by subsequent workers as well (for review see Conant, 1961). Conant (1961) stated that scale pits have been found among all species and sub-species of North American Natrix (Nerodia) for which fresh material is available, although they are apparently absent in many individuals of N. valida and N. kirtlandi (now called Clonophis kirtlandi). Conant also found scale pits on several species of Thamnophis. Thus Conant concluded apical pits were worthless key characters for separating

Natrix from Thamnophis. The failure to find apical pits on museum specimens may not mean that pits are not present on fresh specimens. Conant found that often scale pits are difficult to identify on long preserved material. In the Thamnophis species, often pits were only found on a few scales and not generally distributed as in most Nerodia. Even Regina alleni is reported to possess apical pits on the few scales that have central keels (Rossman, 1963).

Pits observed on Agkistrodon and Nerodia scales were morphologically alike. None was very deep nor possessed specialized surface structures. The presence of apical pits seems to be associated with presence of central keels, and neither is found on strongly fossorial snakes (Jackson and Reno, 1975).

CHAPTER V

SUMMARY AND CONCLUSIONS

The primary aim of this study was to investigate the use of micromorphology in the study of natricine systematics. Watersnakes have been a difficult group for which to establish widely acceptable, enduring classification schemes. Workers in this area desire new characters to supplement traditionally used osteology, gross morphology, hemipenes, and scutellation.

Walls (1942) first applied light microscopy to a study of snake visual cells and since his work Underwood (1970, others) has furthered this application. The present data indicate that while light microscopy provides useful information it fails to provide species distinction. Visual cell patterns and retinae appear to be evolutionarily stable with respect to species group or subfamily categories. At the family level, however, there is more variability.

Walls and Underwood constructed phylogenies of snake visual cell patterns. These differ primarily on which pattern represents the stem pattern from which other patterns radiated. The presence of Type D elements in Nerodia rhombifera supports Underwood's view.

Transmission electron microscopy shows that, ultra-

structurally, there is variation among otherwise similar species. The present data are insufficient to determine the degree and nature of this variation. The ellipsoids in the visual cell inner segments are the most interesting in this regard and more study is needed. Additional study is also needed to determine affects of ontogeny and aging on retinal parameters. Further, analysis of intra-retinal variation is needed to determine if different regions of the snake retina show different cell densities or frequencies.

Scale surface morphology has recently become accessible to detailed study due to the development of the scanning electron microscope. From only three comparative studies (two of which deal with the lizard genus Sceloporus) it is generally reported that scale (oberhautchen) surface patterns are species specific in squamates. The present data do not show such specificity for all species. Scale surfaces of Agkistrodon contortrix and A. piscivorus are very similar. More inter-specific variation exists among the Nerodia and Thamnophis, however.

Phenetic analysis of scale surface patterns shows a high degree of similarity among Nerodia and Thamnophis. The amount of variation within the genus Thamnophis is of equal magnitude to the variation between Thamnophis and Nerodia.

Regina alleni and R. rigida scales are more similar to those of Farancia abacura than they are to scales of Regina septemvittata. Scale surface patterns of Farancia abacura,

Regina alleni, and R. rigida are likely related to fossorial habits.

Scale patterns do not seem to be correlated with any other of the ecological preferences studied.

The major problems encountered with scale study by scanning electron microscopy involve reproducibility. Similar scale patterns are present on different specimens of the same species. Patterns do show varying amounts of erosion, however. Reasonably large numbers of specimens must be available to insure good data from a given species. The scale characters utilized in this study represent actual surface sculpturing and are of taxonomic utility.

Museum collections offer a rich source of material, and the use of such collections should make possible studies of complete species groups.

Additional study is needed to elucidate the affects of ontogeny, aging, and ecdysis cycle on scale patterns.

LITERATURE CITED

Anderson, D. H., S. K. Fisher, and R. H. Steinberg. 1978. Mammalian cones: disc shedding, phagocytosis and renewal. Invest. Ophthalmol. Vis. Sci. 17:117-133.

Bailey, J. R. 1967. The synthetic approach to colubrid classification. Herpetologica 23:155-161.

Baker, R. J., G. A. Mengden, and J. J. Bull. 1972 Karyotypic studies of thirty-eight species of North American snakes. Copeia 1972:257-265.

Bellaris, A. 1970. The life of reptiles. Universe Books, New York. (2 vol.)590pp.

_____ and G. Underwood. 1951. The origin of snakes. Biol. Rev. 26:193-237.

Bogert, C. M. 1940. Herpetological results of the Vernay Angola Expedition. Bull. Amer. Mus. Nat. Hist. 77: 1-107.

Branson, B. A. and E. C. Baker. 1974. An ecological study of the queen snake, Regina septemvittata (Say), in Kentucky. Tulane Stud. Zool. Bot. 18:153-171.

Brown, E. E. 1958. Feeding habits of the northern water snake Natrix sipedon sipedon Linnaeus. Zoologica 43: 55-71.

Burstein, N., K. R. Larsen, and H. M. Smith. 1974. A preliminary survey of dermatoglyphic variation in the lizard genus Sceloporus. J. Herp. 8:359-369.

Carpenter, C. C. 1952. Comparative ecology of the common garter snake (Thamnophis s. sirtalis), the ribbon snake (Thamnophis s. sauritus), and Butler's garter snake (Thamnophis butleri) in mixed populations. Ecol. Monogr. 22:235-258.

Cohen, A. I. 1963. The fine structure of the visual receptors of the pigeon. Exp. Eye Rse. 2:88-97.

Cole, C. J. and T. R. VanDevender. 1976. Surface structure of fossil and recent epidermal scales from North American lizards of the genus Sceloporus (Reptilia,

Iguanidae). Bull. Am. Mus. Nat. Hist. 156:453-513.

Conant, R. 1961. A new water snake from Mexico, with notes on anal plates and apical pits in Natrix and Thamnophis. Amer. Mus. Nov. 2060:1-22.

_____ 1975. A field guide to reptiles and amphibians of eastern and central North America. Houghton Mifflin Co., Boston. 429pp.

Cope, E. D. 1895. The classification of the Ophidia. Trans. Amer. Philos. Soc. 18(new ser.):186-219.

Dawes, C. J. 1971. Biological techniques in electron microscopy. Barnes and Noble, Inc., New York. 193pp.

Denburgh, J. V. and J. R. Slevin. 1918. The garter-snake of western North American. Calif. Acad. Science 8: 181-270.

Dessauer, H. C. 1967. Molecular approach to the taxonomy of snakes. Herpetologica 23:148-155.

Diener, R. A. 1957. An ecological study of the plain-bellied water snake. Herpetologica 13:203-211.

Ditmars, R. L. 1931. Snakes of the world. MacMillan Pub. Co., Inc., New York. 207pp.

Dowling, H. G. 1967. Hemipenes and other characters in colubrid classification. Herpetologica 23:138-142.

_____ 1975. A provisional classification of snakes. Yearbook Herpetol. 1:167-170.

_____ and W. E. Duellman. 1978. Systematic herpetology: a synopsis of families and higher categories. HISS Publ., New York.

_____ and J. M. Savage. 1960. A guide to the snake hemepenis: a survey of basic structure and systematic characters. Zoologica 45:17-28.

Dunn, E. R. 1928. A tentative key and arrangement of the American genera of Colubridae. Bull. Antivenin Inst. Amer. 2:;8-24.

Dunson, W. A. 1975. The biology of sea snakes. Univ. Park Press, Baltimore. 530pp.

Eberle, W. G. 1972. Comparative chromosomal morphology of the new world natricine snake genera Natrix and Regina. Herpetologica 28:98-105.

Foote, R. and J. A. MacMahon. 1977. Electrophoretic studies of rattlesnake (Crotalus and Sistrurus) venom: taxonomic implications. Comp. Biochem. Physiol. 57: 235-241.

Franz, R. 1977. Observations on the food, feeding behavior and parasites of the striped swamp snake, Regina alleni. Herpetologica 33:91-94.

Gans, C. and S. Baic. 1977. Regional specialization of reptilian scale surfaces: relation of texture and biologic role. Science 195:1348-1350.

George, W. E. and H. C. Dessauer. 1970. Immunological correspondence of transferrins and the relationships of colubrid snakes. Comp. Biochem. Physiol. 33:617-627.

Godley, J. S. 1980. Foraging ecology of the striped swamp snake, Regina alleni, in southern Florida. Ecol. Monogr. 50:411-436.

Haines, T. P. 1967. Variations of colubrid skulls, their correlations and their value in taxonomy. Herpetologica 23:142-145.

Hall, R. J. 1969. Ecological observations on Graham's watersnake (Regina grahami Baird and Girard). Amer. Mid. Nat. 81:156-163.

Hardy, L. M. 1971. A comparison of karyotypes of the snake genera Virginia Baird and Girard and Storeria Baird and Girard from northwestern Louisiana. Swanews 2:12.

Hibbard, E. 1975. Eyes and other sense organs of sea snakes. In: The biology of sea snakes, W. A. Dunson (ed.). Univ. Park Press, Baltimore.

_____ and J. Lavergne. 1972. Morphology of the retina of the sea-snake, Pelamis platurus. J. Anat. 112:;25-136.

Hoge, A. R. and P. S. Santos. 1951. Submicroscopic structure of "stratum corneum" of snakes. Science 118:410-411.

Hoffstetter, R. 1968. A contribution to the classification of snakes (review). Copeia 1968:201-213.

Hollyfield, J. G. and S. F. Basinger. 1978. Photoreceptor shedding can be initiated within the eye. Nature 247:794-796.

Horton, D. R. 1973. Lizard scales and adaptation. Syst.

Zool. 21:441-443.

Humason, G. L. 1972. Animal tissue techniques. W. H. Freeman and Co., San Francisco. 641pp.

Jackson, M. K. and H. W. Reno. 1975. Comparative skin structure of some fossorial and subfossorial lepto-typhlopid and colubrid snakes. Herpetologica 31:350-359.

Klauber, L. M. 1935. The feeding habits of a sea snake. Copeia 1935:182.

Maderson, P. F. A. 1964. The skin of lizards and snakes. Brit. J. Herp. 3:151-154.

_____ 1965a. Histological changes in the epidermis of snakes during the sloughing cycle. J. Zool. 146:98-113.

_____ 1965b. The embryonic development of the squamate integument. Acta.Zool. 46:275-295.

_____ 1966. Histological changes in the epidermis of the Tokay (Gecko gecko) during the sloughing cycle. J. Morph. 119:39-50.

_____, B. A. Flaxman, S. I. Roth, and G. Szabo. 1972. Ultrastructural contributions to the identification of cell types in the lizard epidermal generation. J. Morph. 136:191-210.

Malnate, E. V. 1960. Systematic division and evolution of the colubrid snake genus Natrix, with comments on the subfamily Natricinae. Proc. Acad. Nat. Sci. Phil. 112:41-71.

Mao, S. H. and H. C. Dessauer. 1971. Selectively neutral mutations, transferrins and the evolution of natricine snakes. Comp. Biochem. Physiol. 40A:669-680.

Meszler, R. M. 1970. The pit organs of snakes. Appendix II. In: Biology of the reptilia. C. Gans and T. Parsons (eds.). Academic Press, New York. 374pp.

Minton, W. H. and S. K. Salanitro. 1972. Serological relationships among some colubrid snakes. Copeia 1972:246-252.

Mushinsky, H. R. and J. J. Hebrard. 1977a. Food partitioning by five species of water snakes in Louisiana. Herpetologica 33:162-166.

_____ 1977b. The use of time by sympatric water snakes. Can J. Zool. 55:1545-1550.

_____ and M. G. Walley. 1980. The role of temperature on the behavioral and ecological associations of sympatric water snakes. Copeia 1980:744-754.

Oldak, P. D. 1976. Comparison of the scent gland secretion lipids of twenty-five snakes: implications for biochemical systematics. Copeia 1976:320-326.

Pearson, D. D. 1966. Serological and immunoelectrophoretic comparison among species of snakes. Unpubl. Ph.D. Dissertation, Univ. Kansas, Lawrence.

Pedler, C. H. and K. Tansley. 1963. The fine structure of the cone of a diurnal gecko (Phelsuma inunguis). Exp. Eye Res. 2:39-47.

Polyak, S. L. 1957. The vertebrate visual system. Univ. Chicago Press, Chicago.

Preston, W. B. 1970. The comparative ecology of two water snakes, Natrix rhombifera and Natrix erythrogaster, in Oklahoma. Unpubl. Ph.D. Dissertation, Univ. Okla., Norman.

Reimer, W. J. 1957. The snake Farancia abacura: an attended nest. Herpetologica 13:31-32.

Rodieck, R. W. 1973. The vertebrate retina, principles of structure and function. W. H. Freeman Co., San Francisco. 1044pp.

Romer, A. S. 1956. Osteology of the reptiles. Univ. Chicago Press, Chicago. 772pp.

Rossman, D. A. 1963a. Relationships and taxonomic status of the North American natricine snake genera Liodytes, Regina, and Clonophis. Occas. Pap. Mus. Zool. La. State Univ. 29:1-29.

_____ 1963b. Relationships of the Elegans complex of the garter snakes, genus Thamnophis. Year Book Amer. Phil. Soc. 1963:347-348.

_____ 1967. Introduction: symposium on colubrid snake systematics. Herpetologica 23:;37.

_____ and W. G. Eberle. 1977. Partition of the genus Natrix with preliminary observations on evolutionary trends in natricine snakes. Herpetologica 33:34-43.

Roth, S. I. and W. A. Jones. 1967. The ultrastructure and enzymatic activity of the boa constrictor (Constrictor constrictor) skin during the resting phase. J. Ultrastruct. Res. 18:304-323.

_____ 1970. The ultrastructure of epidermal maturation in the skin of boa constrictor (Constrictor constrictor). J. Ultrastruct. Res. 32:69-93.

Rudall, K. M. 1947. X-ray studies of the distribution of protein chain types in the vertebrate epidermis. Biochem. Biophys. Acta 1:549-561.

Smith, H. M. and J. E. Huheey. 1960. The watersnake genus Regina. Trans. Kan. Acad. Sci. 63:156-164.

Sneath, P. H. A. and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman., San Francisco. 573pp.

Sokal, R. R. and C. D. Michener. 1958. A statistical method for evaluating systematic relationships. univ. Kansas Sci. Bull. 38:1409-1438.

Soule, M. and W. C. Kerfoot. 1972. On the climatic determination of scale size in a lizard. Syst. Zool. 21: 97-105.

Stewart, G. R. and R. S. Daniel. 1973. Scanning electron microscopy of scales from different body regions of three lizard species. J. Morph. 139:377-388.

_____ 1975. Microornamentation of lizard scales: some variations and taxonomic correlations. Herpetologica 31:117-130.

Stovall, R. H. 1975. Observations on the micro- and ultrastructure of the visual cells of certain colubrid snakes (Reptilia: Colubridae). Unpubl. M.A. Thesis, Univ. Texas at Arlington, Arlington.

_____ 1976a. Observations on the micro- and ultrastructure of the visual cells of certain snakes (Reptilia, Serpentes, Colubridae). J. Herp. 10:269-275.

_____ 1976b. Ellipsoids of the visual cells of several species of snakes. Texas Soc. Elect. Microscop. Newsletter 7(1).

Strecker, J. K. 1926. On the habits of some southern snakes. Contr. Baylor Univ. Mus. 1926:3-11.

Sutton, D. E. 1969. Fingerprint correspondence of hemoglobin applied to the taxonomy of reptiles. Ph.D. Thesis, Louisiana State Univ. Med. Center, New Orleans.

Tansley, K. and B. K. Johnson. 1956. The cones of the

grass snake's eye. Nature 178:1285-1286.

Taub, A. M. 1967. Systematic implications from the labial glands of the Colubrida. Herpetologica 23:145-148.

Tercafs, R. R. 1963. Transmission of ultra-violet, visible and infrared radiation through the keratinous layer of reptile skin (Serpentes and Sauria). Ecology 44:214-218.

Trapido, H. 1944. The snakes of the genus Storeria. Amer. Midl. Nat. 31:1-64.

Underwood, G. 1951. Reptilian retinas. Nature 167:183-185.

_____ 1966. On the visual-cell pattern of a homalopsine snake. J. Anat. 100:571-575.

_____ 1967a. A contribution to the classification of snakes. Brit. Mus. (Nat. Hist.), London.

_____ 1967b. A comprehensive approach to the classification of the higher snakes. Herpetologica 23:161-168.

_____ 1970. The eye. pp. 1-97. In: Biology of the reptilia. C. Gans and T. Parsons (eds.). Academic Press, New York. 374pp.

Varky, A. 1973. Comparative cranial myology of North American natricine snakes. Ph.D. Thesis, Louisiana State Univ., Baton Rouge.

Voris, H. K. 1969. The evolution of the Hydrophiidae with a critique on methods of approach. Unpubl. Univ. Chicago Thesis, Chicago.

_____ 1977. A phylogeny of the sea snakes (Hydrophiidae). Fieldiana 70:79-169.

Walls, G. L. 1938. The microtechnique of the eye with some suggestions as to material. Stain Technol. 13:69-72.

_____ 1942. The vertebrate eye and its adaptive radiation. Bull. Cranbrook Inst. Sci. 19:1-785.

Yamada, E. T., T. Ishikawa, and T. Hatae. 1966. Some observations on the retinal fine structure of the snake, Elaphe climacophora. 6th Intn'l. Cong. Electron Microscop. Kyoto. II:495-496.

Young, R. W. 1976.. Visual cells and the concept of renewal. Invest. Ophthalmol. 15:700-725.

_____ 1977. The daily rhythm of shedding and degradation of cone outer segment membranes in the lizard retina. J. Ultrastruct. Res. 61:172-185.

APPENDIX A

SCALE SPECIMENS : COLLECTION DATA AND CONDITION

Species	No.	Year	County	State	Condition	No.	Year	County	State	Condition
Agkistrodon contortirx	OSUR 1099	1955	Mayes	OK	?	OSUR 1199	1958	Payne	OK	?
	OSUR 2019	1958	Payne	OK	Bad	OSUR 2855	1964	McCurtain	OK	?
Agkistrodon piscivorus	OSUR 65	no data			Bad	RS 89	no data			Good
Boa constrictor		no data			Good					
Farancia abacura	OSUR 2017	1957	Collier	FL	Good	OSUR 3891	1977	McCurtain	OK	Good
Nerodia cyclopion	OSUR 1996	1959	Lee	FL	Bad	NELS 5229	1965	Lafouche	LA	?
Nerodia erythrogaster	OSUR 2677	1963	Woodward	OK	?	OSUR 3031	1964	Morehouse	LA	?
	OSUR 3145	1941	Sequoyah	OK	Bad	OSUR 3475	1966	Noble	OK	Good
Nerodia fasciata	RS 91	1978	FortBend	TX	Good	OSUR 109	1926	Layfayette	LA	Bad
	OSUR 112	1947	McCurtain	OK	?	OSUR 187	1955	Galveston	TX	Bad
	OSUR 188	1955	Galveston	TX	Bad					

Nerodia rhombifera	OSUR 3664	1976	Sequoyah	OK	Good	OSUR 3866	1977 Noble	OK	Good
	OSUR 3867	1977	Noble	OK	Good				
Nerodia sipedon	OSUR 145	1940	Delaware	OK	Good	OSUR 284	1947 Leflore	OK	Good
	OSUR 491	1952	Adair	OK	Good	OSUR 992	1957 Wyoming	PA	Bad
	OSUR 993	1957	Wyoming	PA	Good/ Bad	OSUR 990	1957 Wyoming	PA	Bad
Regina alleni	OSUR 1782	1959	Collier	FL	Bad	OSUR 1988	1959 Alachua	Fl	Good
	OSUR 1989	1959	Alachua	FL	Good				
Regina grahami	OSUR 209	1954	Payne	OK	Bad	OSUR 1208	1958 King-fisher	OK	?
Regina rigida	OSUR 3104	1962	Ovaohita	LA	Good				
Regina septemvittata	OSUR 895	1957	Franklin	OH	?	OSUR 1011	1957 Franklin	OH	Good
	OSUR 1857	1959	Alle-gheny	PA	Good				
Storeria dekayi	OSUR 364	1954	Payne	OK	Bad	OSUR 3218	1966 Payne	OK	Good

	OSUR 3878	1977	Pushmataha	OK	Good				
Storeria occipitomaculata	OSUR 213	1929	Cheboygan	MI	Bad	OSUR 1206	1958	Cherokee	OK	Good/Bad
Thamnophis butleri	OSUR 84	1938	Washtenaw	MI	Bad	OSUR 1313	1958	Licking	OH	Good
Thamnophis cyrtopsis	OSUR 3424	1966	Santacruz	AZ	?					
Thamnophis elegans	OSUR 1450	1958	Morin	CO	Good	OSUR 1524	1958	Boulder	CO	?
	OSUR 1525	1958	Boulder	CO	Bad	OSUR 1781	1959	Humbolt	CA	Good
Thamnophis eques	OSUR 1879	1959	Cochise	AZ	Good					
Thamnophis marcianus	OSUR 1241	1958	Greer	OK	Good	OSUR 1494	1958	Cochise	AZ	Bad
	OSUR 2117	1959	Throckmorton	TX	Good	OSUR 3884	1977	Kingfisher	OK	Good
	OSUR 3885	1976	Woodward	OK	Good					
Thamnophis proximus	OSUR 3663	1976	Leflore	OK	Good	OSUR 3680	1976	Leflore	OK	?
Thamnophis radix	OSUR 531	1949	Cimarron	OK	Bad	OSUR 598	1949	Cimarron	OK	Good

Thamnophis sauritus	OSUR 1739	1959	Argsbrae	CO	Bad	OSUR 2738	1964	Caddo	OK	Good
Thamnophis sirtalis	OSUR 235	1937	Washte-now	MI	Bad	OSUR 1247	1958	Wyoming	PA	Bad
	OSUR 3886	1977	Payne	OK	Good					
Tropidoclonion lineatum	OSUR 1107	1958	Tulsa	OK	Good	OSUR 2285	1960	Payne	OK	Bad

OSUR = specimen from Oklahoma State University Museum
RS = specimen from R. H. Stovall

APPENDIX B

STUDIES OF THE VISUAL CELLS OF SNAKES

Taxon	Photos EM	Photos LM	Drawings	Authority
BOIDAE				
Epicrates			+	Underwood (1967a, 1970)
Python			+	Underwood (1970)
Tropidophis			+	Underwood (1970); Walls (1942)
Xenopeltis			+	Underwood (1967a, 1970)
COLUBRIDAE				
Abaster				Underwood (1967a, 1970); Walls (1942)
Ahaetulla			+	Underwood (1967a, 1970)
Arizona				Underwood (1967a, 1970); Walls (1942)
Atractus				Underwood (1970)
Boaedon				Underwood (1967b, 1970)
Carphophis				Underwood (1970)
Cemophora			+	Underwood (1967a, 1970); Walls (1942)
Chersydrus			+	Underwood (1970)
Chionactis				Underwood (1967a, 1970)
Coluber	+	+	+	Stovall (1976a); Underwood (1967a, b, 1970); Walls (1942)
Coronella			+	Underwood (1967a, b, 1970)
Crotaphopeltis			+	Underwood (1970)
Dasypeltis				Underwood (1966, 1967a, 1970); Walls (1942)
Diadophis				Underwood (1970)
Dinodon				Underwood (1970)
Dipsadomorphus				Walls (1942)

Dromicus			+	Underwood (1967a, 1970)
Drymarchon				Underwood (1967a, 1970)
Dryophis				Walls (1942)
Elaphe				Stovall (1976b); Underwood (1970); Yamada et al. (1966)
Enhydris			+	Underwood (1966, 1970)
Farancia				Underwood (1967a, 1970); Walls (1942)
Helicops				Underwood (1970)
Heterodon	+			Underwood (1967a, 1968, 1970)
Hypsiglena	+	+	+	Stovall (1976a); Underwood (1967b, 1970); Walls (1942)
Lampropeltis				Underwood (1967a, 1970); Walls (1942)
Leptodeira	+	+	+	Miller and Snyder (1977); Stovall (1975); Underwood (1966, 1970); Walls (1942)
Leptotyphlops			+	Underwood (1951, 1967a, 1970)
Liopholidophis				Underwood (1970)
Lycodonomorphus				Underwood (1970)
Malpolon				Underwood (1967a, 1970); Walls (1942)
Natrix (Nerodia)	+	+	+	Hibbard and Lavergne (1972); Tansley and Johnson (1956); Underwood (1967a, 1970); Walls (1942)
Pareus			+	Underwood (1967a, b, 1970)
Phyllorhynchus				Underwood (1967a, 1970); Walls (1942)
Pseudaspis				Underwood (1970)

Pseudoboa				Underwood (1967b, 1970)
Rhinocheilus				Underwood (1967a, 1970); Walls (1942)
Sibon			+	Underwood (1966, 1967a, 1970)
Storeria				Stovall (1976b)
Telescopus (*Tarbophis*)				Underwood (1966, 1967a, 1970); Walls (1942)
Thamnophis				Stovall (1976b); Underwood (1967a, 1970)
Trimorphodon			+	Underwood (1967a, 1970); Walls (1942)
ELAPIDAE				
Acanthophis			+	Underwood (1967a, 1970)
Denisonia				Underwood (1970)
Elaps				Underwood (1970)
Enhydrina				Hibbard (1975); Underwood (1970)
Hemachatus				Underwood (1967a)
Micrurus				Underwood (1967a, 1970)
Notechis				Underwood (1970)
Pelamis	+	+	+	Hibbard and Lavergne (1972)
Sepedon				Underwood (1970); Walls (1942)
TYPHLOPIDAE				
Typhlops			+	Underwood (1951, 1967a, 1970)
UROPELTIDAE				
Rhinophis				Underwood (1970)
VIPERIDAE				
Agkistrodon			+	Underwood (1967a, 1970); Walls (1942)
Atractaspis			+	Underwood (1970); Walls (1942)

Bitis		+	Underwood (1967a); Walls (1942)
Bothrops			Stovall (1976b); Underwood (1967a, 1970)
Causus		+	Underwood (1967a, 1970); Walls (1942)
Cerastes			Walls (1942)
Crotalus			Underwood (1967a, 1970)
Sistrurus			Underwood (1967a)
Vipera	+	+	Underwood (1967a, 1968, 1970); Walls (1942)

APPENDIX C

RETINAL SPECIMENS : COLLECTION DATA AND COMMON NANE

Species	Common Name	Number	County	State	Year
Agkistrodon contortrix	copperhead	RS82	Harris	Texas	1976
Agkistrodon piscivorus	cottonmouth	RS12	no data	Texas	1974
Farancia abacura	mud snake	RS67	Harris	Texas	1976
Nerodia erythrogaster	yellowbelly watersnake	RS26	no data	Texas	1974
Nerodia erythrogaster	yellowbelly watersnake	RS17	no data	Texas	1974
Nerdoia fasciata	broadbanded watersnake	RS91	Fort Bend	Texas	1978
Nerodia rhombifera	diamondback watersnake	RS27	no data	Texas	1974
Pelamis platurus	sea snake	RS63	no data		
Regina grahami	Graham's watersnake	RS78	Harris	Texas	1976
Storeria dekayi	Dekay's snake	RS77	Harris	Texas	1976
Storeria dekayi	Dekay's snake	RS87	Harris	Texas	1976
Thamnophis proximus	ribbon snake	RS34	no data	Texas	1974
Thamnophis proximus	ribbon snake	RS35	no data	Texas	1974

APPENDIX D

DATA FROM RETINAL COUNTS

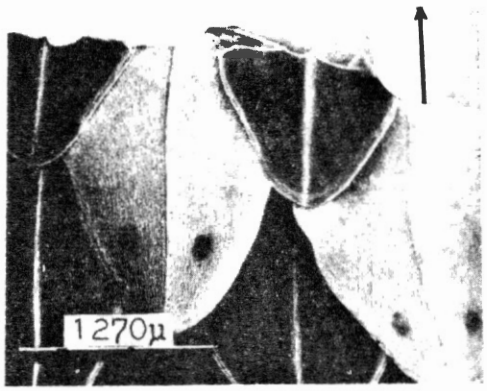
	Agkistrodon contortrix	*Agkistrodon piscivorus*	*Farancia abacura*	*Nerodia erythrogaster*	*Nerodia erythrogaster*	*Nerodia fasciata*	*Nerodia rhombifera*	*Pelamis platurus*	*Regina grahami*	*Storeria dekayi*	*Thamnophis proximus*
specimen	RS82	RS12	RS67	RS26	RS17	RS91	RS27	RS63	RS78	RS77 RS87	RS33 RS34
total cells	584	1544	555	725	716	1389	847	683	845	1253	907
% receptors	50.0	44.3	23.2	13.6	13.3	13.0	15.0	23.4	16.0	12.1	12.3
% interneurons	45.5	49.0	66.3	77.9	77.2	77.6	76.6	66.3	73.8	78.7	80.3
% ganglion c.	4.3	6.6	10.5	8.4	9.5	9.4	8.4	10.2	10.2	9.3	7.4
% Type A	15.3		44.7	76.5	67.4	89.1	67.0	83.0	64.7	92.8	81.3
% Type B	2.5		9.6	7.1	24.4	3.1	17.0	7.8	17.2	6.3	9.9
% Type C	82.2		45.6	16.5	8.1	7.8	9.4	9.1	18.1	0.9	8.8
% Type D							6.6				

APPENDIX E

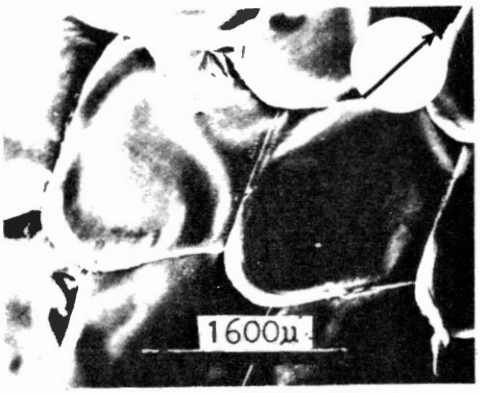
GLOSSARY OF SCALE CHARACTERS

1. Overall Shape
   refers to the shape formed by an outline of the scale perimeter as viewed from directly above

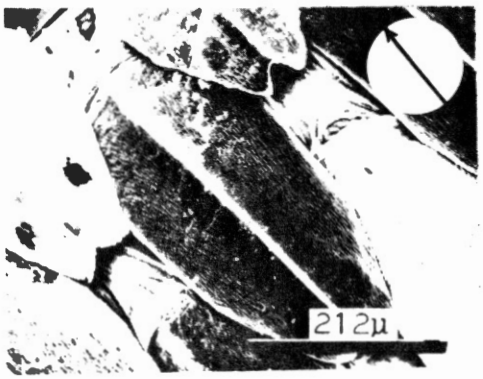
   1-1 Agkistrodon-like
   scales approximately twice as long as wide (at widest point), smoothly taper to a bluntly rounded tip



   1-2 Farancia-like
   scales distinctly rhomboidal in shape and bluntly tapered



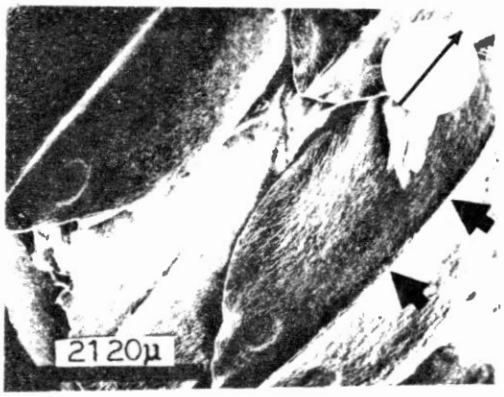
   1-3 Nerodia-like
   scales approximately twice as long as wide but tapering essentially from the posterior 1/3 to end; apical margin V-shaped in profile



2. Central Keel
   refers to a prominant ridge running parallel with the long axis of the scale, in the center of the dorsal (exposed) surface; readily visible to gross observation when present

   2-1 Present

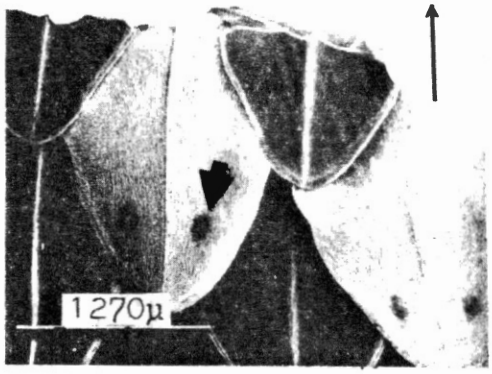
   2-2 Absent



3. Apical Pits
   refers to rounded, pit-like depressions on either side of the central keel posteriorly

   3-1 Present

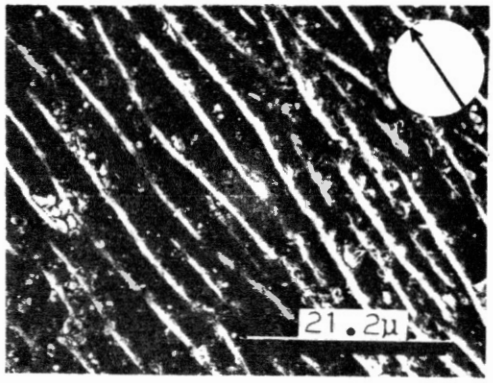
   3-2 Absent



4. Longitudinal Ridges
   refers to linear striations which run parallel to the long axis of the scales but are not nearly as prominent as the central keel; only slightly visible (if at all) to gross observation

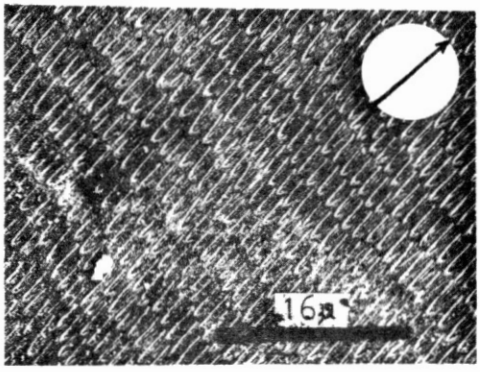
   4-1 Present

   4-2 Absent

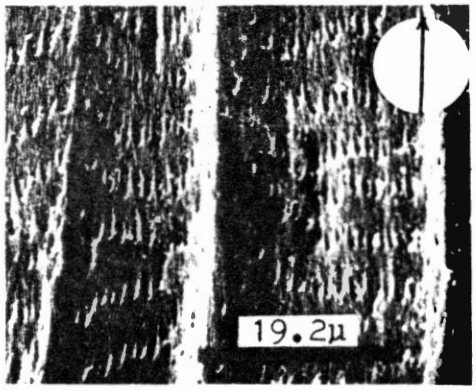


5. Perpendicular Spikes
surface covered with horizontal units or rows from which spike-like projections extend posteriorly; spikes are oriented parallel to the long axis of the scale

5-1 Spikes of adjacent rows interdigitate



5-2 Spikes of adjacent rows overlap

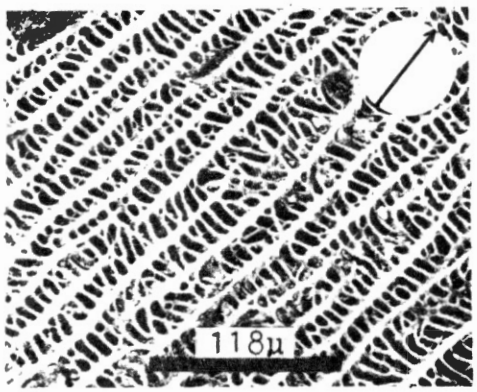


5-3 Absent

6. Inter-ridge Lattice Work
refers to the presence of a complex network of small ridges that form irregular closed spaces to give a net like appearance; when present lattice fills the spaces or troughs between longitudinal ridges

6-1 Present

6-2 Absent



7. Polygonal Units
scale surface appears to be covered by irregular polygonal units; polygonal units may give rise to the longitudinal ridges (character 4) by chevron-like posterior projections which overlap with similar extensions from adjacent polygonal units

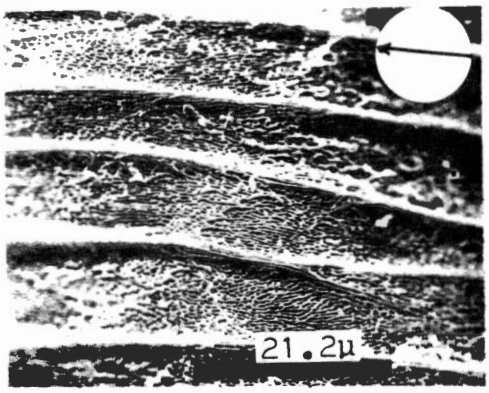
7-1 Present

7-2 Absent

8. Anastomosing Ridges
refers to a surface pattern of numerous small ridges that form a network; ridges are distinctly smaller than longitudinal ridges (character 4); the larger longitudinal ridges often appear as superimposed surface folds on the pattern of the more delicate ridges

8-1 Present

8-2 Absent



VITA

Randall Howard Stovall

Candidate for the Degree of

Doctor of Philosophy

Thesis: APPLICATIONS OF MICROMORPHOLOGY TO NATRICINE SYSTEMATICS (SERPENTES, COLUBRIDAE): VISUAL CELLS AND SCALES

Major Field: Zoology

Biographical:

Personal Data: Born in Norman, Oklahoma, 3 February, 1951, the son of Mr. and Mrs. Jack W. Stovall.

Education: Graduate from Putnam City High School, Oklahoma City, Oklahoma, in May, 1969; attended the University of Oklahoma, Odessa College, Midland College, and received Bachelor of Science degree in Biology from the University of Texas at Arlington in 1973; received Master of Arts in Biology from the University of Texas at Arlington in 1975; attended the University of Texas Health Science Center at Houston before entering Oklahoma State University in 1978; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1982.

Professional Experience: Graduate Teaching Assistant, Department of Biology, the University of Texas at Arlington, 1973-75; Graduate Research Assistant, the University of Texas Graduate School of Biomedical Science (Houston), 1975-77; Instructor of Biology, North Harris County College, 1977-78; Graduate Teaching Assistant, School of Biological Sciences, Oklahoma State University, 1978-79; Assistant Professor of Biology, Northwestern Oklahoma State University, 1979-80; Instructor of Biology, Pasco-Hernando Community College, 1980-present; Program Biologist and Editor, Environmental Studies Center, Hillsborough Community College, 1981-present.