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Relationships of epidermal morphology and breeding behaviors in pebble nest-building minnows (Pisces: Cyprinidae)

William R. McGuire

RELATIONSHIPS OF EPIDERMAL MORPHOLOGY AND BREEDING BEHAVIORS IN PEBBLE NEST-BUILDING MINNOWS (PISCES: CYPRINIDAE)

by

WILLIAM REED McGUIRE

APPROVED

COMMITTEE CHAIRMAN

COMMITTEE MEMBERS

EXAMINING FACULTY:

Relationships of Epidermal Morphology and Breeding Behaviors in Pebble Nest-Building Minnows (Pisces: Cyprinidae)

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WILLIAM REED M^{C}GUIRE

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Abstract

The objective of this study was to determine if epidermal morphology corresponds with reproductive behavior in cyprinid fishes. Representative species of nest-building fishes (*Nocomis leptocephalus, Semotilus atromaculatus, Exoglossum maxillingua*) and non nest-building fishes (*Campostoma anomalum* and *Clinostomus funduloides*) were studied. Light and scanning electron microscopy were used to examine epidermal morphology and keratin distribution of skins from the snout, cheek, mandible, anterior dorso-lateral trunk, and caudal peduncle of breeding males of nest-building species and were compared to those in non nest-building cyprinid species. Previous frame-by-frame analysis of video tapes of reproductive activities of each species and literature accounts were used to identify and describe breeding behaviors.

The location and structure of keratinized regions in the epidermis of these cyprinid fishes is species-specific. The distribution of keratin and the form in which it occurs correlates with four primary aspects of reproduction: substrate modification (nest-building or pit digging), spawning, agonistic behaviors (combat and/or aggressive displays), and ornamentation (species and sex identification).

Introduction

There have been numerous investigations on the epidermis of fishes (Henrickson and Maltoltsy, 1968a, b, and c; Whitear, 1970; Harris, 1975; Whitear, 1984). Most studies of species in the family Cyprinidae have focused on a particular epidermal feature: Reed et al. (1972) and Pfeiffer (1978) studied unicellular glands responsible for fright reactions among cyprinid fishes; Roberts (1982) described horny projections arising from single epidermal cells as unculi; Egami and Nambu (1961) stated that innervation of fin rays bearing tubercles of male *Oryzias latipes* was greater than that in fin rays of females, which lack tubercles.

Development of breeding tubercles (mounds of epidermal keratinized cells) during spawning season is a feature of the epidermis of several cyprinids. Fowler (1912) and Denoncourt (1969), among others, examined tubercle distribution. Branson (1962) and Wiley and Collette (1970) described tubercle anatomy. Wiley and Collette (1970) used differences in tubercle distribution and structure to support evidence for phylogenetic relationships among ordinal groups of cyprinids.

Gross morphological characteristics of sexually dimorphic tuberculate fishes have been related to aspects of reproductive behavior (Reighard, 1903; 1910; 1920; Raney, 1940) and linked to endocrine control (Branson, 1962; Egami and Nambu, 1961). Reighard (1910) stated that tubercles displayed on male pebble nest-building fish are important in agonistic behavior. Other investigators related tuberculate pectoral fins to spawning clasps, wherein the male holds the female during oviposition and gamete release (Hubbs, 1942; Maurakis et al., 1991a).

The objective of this investigation is to determine if

epidermal morphology (e.g. keratin distribution) of representative species of nest-building cyprinid genera corresponds to the specific reproductive behaviors, nest-building, spawning, ornamentation, and agonistic behaviors.

In contrast to previous investigators, who described reproductive behaviors of cyprinids based on field notes, this study identifies those behaviors with a frame-by-frame analysis of videotapes. Using two methods of microscopy, my study focuses on male cyprinid fishes in the genera *Nocomis*, *Semotilus*, and *Exoglossum* that use their jaws in moving gravel to modify the stream bed for the construction of pebble nests for spawning. Other cyprinids (*Campostoma anomalum* and *Clinostomus funduloides*), called nest associates, that do not construct nests but use them for spawning are included for comparison.

Specimens Examined

Several mature males of each species, other than those listed in the text, were examined (Appendix) to ascertain that keratinized tissue patches were consistent in location among individuals within a species. Specimens from the following collections were used to describe the epidermal morphology in reproductively active males of the species in this study. The state, drainage, collection number (WSW = William S. Woolcott and EGM = Eugene G. Maurakis), county, location, and date for each species are:

Nocomis leptocephalus. Virginia: New, EGM-VA-256, Carrol Co., tributary of Big Island Creek, Co. Rt. 673, 0.8 km S of US 221, 2 June 1990.

Semotilus atromaculatus. Maryland: Potomac, EGM-MD-246, Washington Co., tributary of Little Antietam Farm Creek on property of Brookview Hospital, Leitersburg Rd. at Jct. with Durberry Rd., 2 May 1990.

Exoglossum maxillingua. Virginia, James, EGM-VA-260, Craig Co., Johns Creek, Co. Rt. 632 at Maggie, 13 June 1990.

Campostoma anomalum. Virginia: James, WSW-VA-392, Bath Co., Hot Springs Run at bridge on Co. Rt. 687 about 0.16 km N of Co. Rt. 687 and Co. Rt. 605, 21 May 1990. New, EGM-VA-252, Craig Co., Sinking Creek, St. Rt. 42 about 1.6 km S of Jct. with Co. Rt. 626, 16 May 1990.

Clinostomus funduloides. Virginia: Roanoke, EGM-VA-259, Montgomery Co., North Fork of Roanoke River, Co. Rt. 785 near Jct. Co. Rt. 1035, 4.8 km E of Blacksburg, 2 June 1990.

Materials and Methods

Fishes were collected with a Smith-Root type 4 pulsed D.C. electroshocker, preserved in 10% formalin or Bouin's fluid, and cataloged into the University of Richmond Fish Collection. After fixation, specimens were rinsed in tap water for 48 hours. Sex was verified by examination of gonads. Fishes were weighed and measured to standard length(SL). The skin was removed from the right side of each fish. Skins were dehydrated in step series to 70% ethanol and stored. Two samples of epidermis from the snout, cheek, mandible, anterior dorso-lateral trunk, pectoral fin base, and midlateral caudal peduncle regions(Fig. 1) were examined from each specimen of pebble nest-building species. Comparisons were made with samples from *C. anomalum* (pit excavator) and *C. funduloides*

(nest associate, a species that spawns over a nest but does not contribute to its construction).

Skin samples used for scanning electron microscopy (SEM) were dehydrated to absolute ethanol and lyophilized in a Labconco Freeze Dry 3, Model 75200, mounted on 15 mm Hitachi stubs, and stored in a dessicator. Three 6 micrometer gold/palladium coatings were applied with a Hummer Type VII sputter coater. Stubs were rotated 60^{o} between applications to ensure an even coating. Surface observations were made at 15 kV with a Hitachi S-2300 Scanning Electron Microscope. Micrographs were made with 10.2 x 12.7 cm Kodak Tri-X Pan Professional Film developed in Kodak HC-110.

Skin tissues for light microscopy (LM) were dehydrated to absolute ethanol, cleared with xylene, and embedded in Paraplast II™. Ten micrometer sections, cut with an American Optical rotary microtome, were mounted with gelatin fixative on hand-cleaned slides. Initially, an albumin fixative was used but was discontinued as it rendered excessive background staining. Slides were stained with Heidenhain's rapid step method of Mallory's collagen stain (Cason, 1950) as recommended by Wiley and Colette (1970). Additional staining was done with hematoxylin and eosin according to methods of Humason (1967). Examinations of stained slides were made with an Olympus Phase Contrast microscope with an ocular micrometer calibrated with an American Optical stage micrometer. Micrographs were made with a Nikon Labophot microscope with 35 mm Kodak Ektachrome film, and Kodak Technical Pan Film developed with HC-110. Cell nomenclature follows Henrikson and Maltoltsy (1968a, b, and c)

and Whitear (1970,1984)(Fig. 2).

ANOVA (SAS, 1985) and Duncan's multiple range test (Steel and Torrie, 1980) were used in the analyses of differences ($p \leq 0.05$) in epidermal thickness, number of epidermal and tubercle cells, tubercle cap thickness, and total tubercle size among the species (Fig. 2).

Descriptions of substrate modification (i.e., nest-building and pit excavating), spawning, and agonistic behaviors are based on literature accounts, personal observations, and previous review of videotapes of fishes (Appendix) studied in streams in North Carolina and Virginia from 1986 to 1992. Field videos were recorded above the surface of the water with a Charge Coupled Device (CCD) solid state image sensor video camera equipped with a polarizing filter to minimize reflected light during daylight hours. In the laboratory, 13.3 video-recording hours were replayed frame by frame (30 frames/sec) to identify behaviors of each species where literature accounts were either unavailable or ambiguous.

Results

Results of the epidermal morphological study are presented by body region (Fig. 1).

Snout.- The snout region is the exterior smooth epidermis between the anterior margin of the maxilla and a line that extends between the eyes across the top of the head. Nostrils and the infraorbital canal are included.

Tubercles were found on the snout of all adult males of each species except *E. maxillingua* (Table 1). Those of *N. leptocephalus* and *C. anomalum* were the largest in any of the

species (2.1 and 1.7 mm high, respectively). Tubercles of both species were hard rounded mounds of polygonal keratinized cells (Fig. 3a), some of which appeared worn or damaged (Fig. 3b). *Semotilus atromaculatus* tubercles were wide-based short mounds of keratinized polygonal epidermal cells (Fig. 4). *Clinostomus funduloides* had small conical tubercles (Fig. 5).

Skins of tuberculate species were of two distinct morphological forms. In *N. leptocephalus, S. atromaculatus,* and *C. funduloides,* localized increased mitotic activity in the epidermis produces an aggregation of cells, the surface ones of which eventually keratinize and form a cap of keratin resulting in a tubercle (Fig. 6).

A second type of tubercle, restricted to *C. anomalum,* was comparatively large, composed of dense hard keratin, and deeply rooted in the epidermis. These tubercles always were located in epidermal pits that extended into the dermis, and unlike keratinized regions of other species, there were no dermal protrusion (i.e., dermal pilli) in the pits (Fig. 7). Approximately one third of mature tubercles had a small envelopment of epidermal cells in the base (Fig. 7). There are no data on mitotic activities or pre-keratin filament production as tubercles were not seen in developmental stages.

Thickness of tubercle caps varied significantly among species (Table 1). Those of *S. atromaculatus* and *N. leptocephalus* had a significantly greater number of cells than did *C. funduloides.* Density of keratin in all regions for *C. anomalum* obscured cellular structure and precluded measurement and analyses of keratin cap thicknesses and cell

counts. Nest-building species had significantly fewer numbers of cells in the epidermis than did *C. funduloides* and *C. anomalum*. The epidermis of *N. leptocephalus* and *C. anomalum* was significantly thicker than that those in other species.

With SEM the epidermis of *E. maxillingua* was a smooth surface of squamate cells interspersed with neuromasts (distinguished from surrounding epithelial cells by the presence of a stalk-like cupola and basal sensory cells). Some neuromasts appeared as mounds covered by a single layer of squamate cells. An occasional neuromast occurred as a deep crater (without a cell covering) with the thread-like cupola converging into its center (Fig. 8).

With light microscopy, skin surface cells of *E. maxillingua* appeared partially cuboidal. Neuromasts were observed in cross sections. Unlike the skin in this region of the other species, keratin was absent and unicellular glands were abundant (sacciform cells, club cells, and serous goblet cells).

Cheek - This region, below the orbit, extends from the posterior margin of the mandible to the posterior periphery of the preopercle. Only *C. funduloides* had tubercles on the cheek.

Under SEM, the non-keratinized epidermis of each species was like that of their snouts. Neuromasts were abundant, many of which resembled unculi described as horny projections arising from single cells in the skins of *C. anomalum, N. leptocephalus*, and *S. atromaculatus* by Roberts (1982).

With LM, the epidermis shared most epidermal histological

characteristics of the snout (Table 2). The epidermis of all species had columnar basal cells except *C. funduloides*, which had cuboidal basal cells. *Nocomis leptocephalus* and *C. anomalum* had squamate surface epithelia, *C. funduloides* incomplete squamation, and *E. maxillingua* polygonal cells. Surface epithelial tissue (1-3 cells thick) of *S. atromaculatus* was composed of nucleated squamate cells containing keratin filaments. *Nocomis leptocephalus* had nucleated non-keratinized squamate and semi-squamate (with some cytoplasm) cells. With SEM, these cells resembled unculi, giving a rough appearance to the surface of the skin.

As with snout epidermis, unicellular glands were abundant in *E. maxillingua*. Serous and sacciform cells were present in *N. leptocephalus*, and serous only in *S. atromaculatus*. Subtubercular cells in *C. funduloides* were actively dividing when fixed as all mitotic stages were represented (Fig. 9). Dermal pilli extended into each tubercle of this species as well as into the non-keratinized surface areas of the epidermis. Birefringence (double refraction of plane polarized light by tissues) indicated that the epidermis in the cheek region of *C. anomalum* was an acellular cuticle of non-uniform thickness (Fig. 10).

Nocomis leptocephalus, with larger epidermal cells, had significantly thicker epidermis than the other species, whereas *C. anomalum* had significantly more cells in the epidermal layer (Table 2). *Nocomis leptocephalus* and *C. funduloides* had significantly more epidermal cells than either *E. maxillingua* or *S. atromaculatus*.

Mandible - The skin on the anterior interior border of

the mandible and the external skin surfaces immediately ventral and posterior to the tip of the mandible characterize this region. Samples were obtained by separating the dentaries at the symphysis. Dorso-ventral sectioning of the dentary provided histological sections that were perpendicular to a tangent of the curvature of the mandible.

Clinostomus funduloides, the only species with tuberculation on the outer skin surfaces of the mandible, had tubercles approximately the size of those on the snout (Fig. 11). All mature males of nest-building species and *C. anomalum* had smooth outer mandibular skin.

Only the pebble carrying nest-builders, *N. leptocephalus*, *S. atromaculatus*, and *E. maxillingua* had keratin on the interior surface of the mandible (Fig. 12). The pit digger, *C. anomalum*, had keratin (5-8 cell layers thick) on the exterior ventral epidermis of the mandible. Squamate epidermis with serous goblet cells and neuromasts dispersed throughout, covered the outer surfaces of the mandible of *C. funduloides*.

Nocomis leptocephalus, *S. atromaculatus*, and *E. maxillingua* had collagenous dermal pilli that extended vertically into the interior mandibular epidermis (Fig. 13). In *C. anomalum*, dermal pilli were long strands of collagenous dermis (paralleling the long axis of the fish) extending beneath and supporting the exterior keratinized mandibular epidermis. The collagen with the keratin results in a rigid lip on the anterior margin of the mandible, making an efficient digging tool (Fig. 14).

Basal cells in *N. leptocephalus* were typically short and mitotically active when preserved. *Semotilus atromaculatus* had

an intermediate layer of prickly cells with a higher degree of stratification in the epidermis than was in skins of other species. Also, unlike other species, *S. atromaculatus* had supporting plugs of keratinized cells that extended halfway down into the epidermis (Fig. 12). Presence of acidophilic substances in the epidermis of *E. maxillingua* indicated that all cells, except basal cells, contained keratin filaments (Fig. 15).

The mandibular epidermis of *N. leptocephalus* was significantly thicker and had a greater number of cells than did that of other species. In turn, epidermal thicknesses in *S. atromaculatus* and *E. maxillingua* were significantly greater than those in *C. anomalum* and *C. funduloides*. Numbers of lip epidermal cells in *C. funduloides* were significantly lower than those in other species (Table 3).

Pectoral Fin - Dorsal surfaces of the right pectoral fin ray bases were examined in each species. All species except *E. maxillingua* had medially recurved tubercles arranged in a single or double row along the dorsum of each ray (Fig. 16).

All tuberculate species had mitotically active (when prepared), non-squamate surface epithelial cells. Basal cells were cuboidal. Excepting *E. maxillingua*, keratin filaments were present in the epidermal cells of all species but only in *C. funduloides* were the basal cells involved. Dermal pilli supported keratinized tubercles in *N. leptocephalus;* tubercles of other species were without supportive dermal pilli. Dermal pilli were present, however, in non-keratinized skin of *C. funduloides*. *Campostoma anomalum* was unique as there was no dermal tissue between the epidermal basement membrane and the

fin rays (Fig. 17). Neuromast cells were characteristic of *E. maxillingua* epidermis. Serous goblet cells were present in all species except *C. anomalum*.

The epidermis of the pectoral fin of *N. leptocephalus* was significantly thicker than that of *S. atromaculatus* (Table 4). Average number of epidermal cells in the fin of *C. anomalum* was significantly greater than that in all species except *N. leptocephalus*.

Average thickness of the keratinized cap in *C. funduloides* was significantly less than that in other species. Average number of keratinized epidermal cells was significantly different among species (Table 4).

Anterior Dorso-lateral Trunk - This region included the scaled dorso-lateral area posterior to the cranial occiput and the posterior margin of the opercle, dorsal to the lateral line and anterior to the base of first dorsal fin rays.

Only *C. anomalum* and *C. funduloides* were tuberculate. Hemispherical tubercles of *C. anomalum* were in centers of trunk scales (Fig. 18); acutely conical tubercles in *C. funduloides* were on posterior margins of scales (Fig. 19).

All specimens except *E. maxillingua*, had a surface epithelia completely covered with non-nucleated squamous cells. Those of *E. maxillingua* were nucleated. The epidermis of scales of all species, except *C. anomalum*, had an abundance of serous goblet cells where the epidermis of the preceding scale overlapped that of the following scale (Table 5). The aglandular epidermis of *C. anomalum* was thick($\bar{x}$ = 0.34 mm), especially at the focus of the scale, and was characterized by dermal pilli and deeply rooted tubercles (Fig. 20). Club cells

were present in *C. funduloides*, *N. leptocephalus*, and *E. maxillingua*. The greatest degree of cell stratification occurred in the skin of *N. leptocephalus* (Fig. 21).

There were no significant differences in epidermal thicknesses among species. *Campostoma anomalum* and *N. leptocephalus*, however, had significantly greater numbers of cells in the epidermal layer than did *S. atromaculatus*, which in turn, had significantly more cells than *C. funduloides* and *E. maxillingua* (Table 5).

Caudal Peduncle - The narrow region of the trunk anterior to the proximal end of the caudal fin and posterior to the dorsal fin defines the caudal peduncle.

Tubercles were present on *C. anomalum*, *C. funduloides*, and *S. atromaculatus*. Tubercles of non-nest-builders were like those on their anterior dorso-lateral trunk. *Semotilus atromaculatus*, which had a smooth anterior dorso-lateral trunk skin, had tubercles on the posterior margins of scales. In *S. atromaculatus* and *C. funduloides* tubercles were centered in a broad keratinized area supported by an aggregation of irregularly shaped cells. *Campostoma anomalum* exhibited the same tubercle morphology as described for its anterior dorso-lateral trunk. Tubercle cap thickness and number of keratinized cap cells in *S. atromaculatus* was significantly greater than in *C. funduloides*. Maximum tubercle size (height) of *S. atromaculatus* tubercles was twice that of *C. funduloides* (Table 6).

Histologically prepared epidermis of all species had surface squamate and cuboidal basal cells. A variety of mitotic figures indicated there was active cell division in the

skin of all species except *N. leptocephalus*. Club cells were present in *N. leptocephalus* and *E. maxillingua*.

Campostoma anomalum had a significantly greater epidermal thickness than all other species; and a significantly greater number of epidermal cells than did *S. atromaculatus* and *N. leptocephalus*, which were significantly greater in number than those of *C. funduloides* or *E. maxillingua* (Table 6).

Discussion

The location and structure of keratinized cells in the epidermis of pebble nest-building, pit digging, and nest associate cyprinids is species-specific (Tables 1-7). In these fishes, the distribution of keratin and the form in which it occurs are involved in four main aspects of reproduction: substrate modification (nest-building or pit digging), spawning, ornamentation (species and sex identification), and agonistic behaviors (combat and/or displays of aggression) (Table 8).

Snout.- In all species except *E. maxillingua*, which is non-tuberculate, snout tubercles are associated with agonistic behaviors. In *N. leptocephalus*, the male butts his head laterally with heads of other males to establish and maintain his territory among other males over a nest. Male *N. leptocephalus* also engage in a circle swim, where the dominant male and an equal-sized challenging male swim head to tail in a circle and whorl over a nest (Maurakis et al., 1991a). Videotape analysis revealed that the large pointed keratinized tubercles of the antagonists damage heads and caudal peduncles during head butt and circle swim behaviors (Appendix, tapes

EGM-NC-211, EGM-VA-202).

Unlike *N. leptocephalus*, male *S. atromaculatus* butt their tuberculate heads in a frontal direction during parallel swims, frequently resulting in injury (Reighard, 1910; Maurakis and Woolcott, 1989a; and personal observations).

Male *C. anomalum* defend spawning pits during construction with parallel swims and "swing and butt" behaviors (Miller, 1962, Maurakis et al., 1991b). The "swing and butt," where a male rotates his heavily tuberculate snout and body laterally and directly toward his aggressor, are movements that result in injury to another male upon contact.

No recorded accounts of aggression in *C. funduloides* are available. Aggressive behavior is described from review of videotapes (Appendix, tapes EGM-VA-221; EGM-VA-289) and compared to descriptions by Koster (1939) for *Clinostomus elongatus*, a closely related species (Deubler, 1955). Combat in *C. funduloides* consists of threats of aggression and snout contact with their acutely pointed tubercles that frequently cause injury. According to Koster (1939) displays of aggressive behavior, for the sole purpose of territorial defense, ceases after spawning has begun.

Smooth tubercles in the nest-builders may also serve as ornamentation. According to Wiley and Collette (1970), distribution and size of tubercles may be involved in species identification, sex and an indication of physiological readiness of mates.

Non-tuberculate nesting male *E. maxillingua* do not perform extended combat with other males. Contact is limited to short parallel swims and lunges at challenging fish as described by

Maurakis et al. (1991b). This relatively thin-skinned species is covered with unicellular secretory glands like those containing alarm substances responsible for flight and fright reactions (Pfeiffer, 1977; 1978; Reid, 1972). Whereas the other nest-building species have nest associates during the spawning season, Maurakis et al. (1991b) report that they have not seen them over the nests of *E. maxillingua* and its cogenor, *Exoglossum laurae,* in Virginia. Possibly, alarm reaction substances in the unicellular secretory glands of *E. maxillingua* are responsible for their reduced aggressive behavior over nests.

Cheek - *Clinostomus funduloides* was the only species with tuberculation on the cheek region, which probably serves in ornamentation display. Absence of cheek tubercles in nest-building species is associated with the absence of contact during combat, spawning, and nest-building. However, the layer of keratinized epidermal cheek cells in *S. atromaculatus* may protect this skin area during pebble excavation. It is the only species whose entire lower head, including the cheek, is forced into the substrate during excavation of pebbles.

Mandible - Except for *C. funduloides* (nest associate), distribution of mandibular keratin is associated with preparation of the spawning substrates. Male pebble nest-building fishes use their jaws to excavate and move rocks and gravel to form spawning nests where they spawn with females (Woolcott and Maurakis, 1988; Maurakis et al., 1990, 1991a, and 1991b). Mature males of all pebble nest-building cyprinid species in this study have keratin (anchored by vertical dermal pilli) on interior mandibular epidermal surfaces. This

epidermis, subject to abrasion while males excavate and transport stones with their jaws, functions to protect the mouth epidermis from damage. Other internal mandibular epidermal characteristics in pebble nest-builders are species-specific. For example, in *N. leptocephalus* the thick epidermis was keratinized only on the surface; in *E. maxillingua* all of the post basal epidermal cells were keratinized; and in *S. atromaculatus* supportive plugs of keratinized cells anchor the keratinized layer as they protrude into the non-keratinized epidermis.

Unlike pebble nest-building males, male *C. anomalum* (pit digger) have a thick external mandibular epidermis with extensive keratin, anchored by numerous longitudinal strands of dermal collagen. Rather than lifting and transporting stones, a breeding *C. anomalum* excavates a pit for spawning by pushing stones aside with the exterior skin surfaces of its mandible (Miller, 1962). Videotape accounts of males excavating their pits corroborate Miller's statement that inner mandibular regions do not contact the substrate (Appendix, tapes WSW-VA-383; WSW-VA-381; EGM-VA-225).

Distribution of external mandibular keratin in *C. funduloides*, like its function in the cheek region, is related to spawning and agonistic combat (Appendix, tapes EGM-VA-221; EGM-VA-289). Male *C. funduloides* (nest associate), without keratin on exterior mandibular epidermal surfaces, do not build nests nor dig spawning pits (Deubler, 1955; personal observations). Keratin tubercles on the external mandibular surface probably are associated with limited combat with other males as described by Koster (1939) for *C. elongatus*. They

also may serve as ornamentation for species and sex recognition according to Fowler (1912).

Pectoral Fin -Pectoral fin tuberculation, present in all species except *E. maxillingua*, is associated primarily with spawning. In species of *Nocomis* and *Semotilus*, pectoral fin tuberculation assists the male in holding the female during different maneuvers involved in a successful spawn (Reighard, 1903; Fowler, 1912) over the area where gametes are released [(i.e., pit/ridge interface in nests of *Semotilus* (Maurakis et al., 1990), pit on upstream slope of mound nests in *Nocomis* (Maurakis et al., 1992)]. Reighard (1910) stated that, at the height of a spawning clasp in *S. atromaculatus*, the upper surface of the male's pectoral fin is pressed against the female's ventral surface, serving to momentarily hold her as gametes are released. In *N. leptocephalus*, the pectoral fin serves a similar function during the spawning clasp. The clasp involves a lateral push of the male's body against the female's body as she is held by his pectoral fin during gamete release (Maurakis et al., 1991a). *Exoglossum maxillingua*, without pectoral fin tubercles, uses his entire body to force the female into the substrate of the nest where eggs and sperm are released (Maurakis et al., 1991b).

Unlike pebble nest-building males, *C. anomalum* males do not clasp when spawning. Instead, review of videotapes (Appendix, tapes WSW-VA-383; WSW-VA-381; EGM-VA-225) shows that as a female moves into the the spawning pit, one to five males align themselves laterally to the female. She will then be turned so that her body is perpendicular to the current as the males press her into the substrate with their pectoral girdles

and fins. These observations concur with Miller (1962) who analyzed breeding behavior of *C. anomalum* with movies.

There are no spawning accounts for *C. funduloides*, but according to Koster (1939), *C. elongatus* does not have a clasp. Spawning occurs after a run when a female may go into the pit of a *S. atromaculatus* nest where she spawns with a male. Video accounts of *C. funduloides* over *Semotilus* nests show a similar spawning maneuver in *C. funduloides* (Appendix, tapes EGM-VA-221; EGM-VA-289). Like *C. anomalum*, pectoral fin tubercles may help male *C. funduloides* to compete for position during the spawning run with the female and then hold her momentarily as gametes are released. Wiley and Collette (1970) and Foster (1967) support the hypothesis that pectoral fin tubercles play a tactile role during spawning. Extension of the tuberculate pectoral fins may signal the female that the male is in position to clasp.

Anterior Dorso-lateral Trunk - None of the nest-building fishes in this study had tubercles on the anterior dorso-lateral trunk.

The tubercles on the anterior dorso-lateral trunk of *Campostoma* and *Clinostomus* are used for both combat and breeding. A writhing sideways movement of *C. anomalum*, similar to the parallel swim, has been described as agonistic action between competing males by Miller (1962). He observed a swim in which there was contact between the dorsal trunk tubercles of male combatants. Frame by frame analysis of video tapes show that actually there are two distinct motions in these parallel swims (Appendix, tapes WSW-VA-383; WSW-VA-381; EGM-VA-225). The trunk contact described by Miller originated

posterior to the opercle and continued in a sinusoidal wave toward the caudal peduncle as the two males roll their bodies against each other. The second motion was a slap of the caudal peduncle directed at any portion of the body of another male. These two motions independent of each other did not occur in any particular sequence.

The anterior dorso-lateral trunk tubercles of male *C. anomalum* are associated with maneuvers, like those described for his pectoral fin, to secure a position aside the female during gamete deposition. The extreme epidermal thickness and deeply rooted horny tubercles on scaled regions of *C. anomalum* serve not only as mechanical abrasives, but as protective shields from injury during combat and displacement maneuvers.

Caudal Peduncle - *Semotilus atromaculatus* was the only nest-building species with caudal peduncle tuberculation. Sabaj (1992) stated that during the spawning clasp the male's caudal peduncle prevents the female from drifting downstream by anchoring it to the substrate during gamete deposition. The extra purchase afforded by the abrasive action of the rounded caudal peduncle tubercles not only hold the female in position, but helps to increase the constrictive pressure of the male on the female when the gametes are released.

Campostoma anomalum males use their tuberculate caudal peduncles in agonistic behavior and spawning. Males defend their space (i.e., spawning pit) and position aside a female by using their caudal peduncles to slap other male *C. anomalum* from the pit.

Male *C. funduloides* use their caudal peduncles much in the same manner as in *C. anomalum*. Although there are no

literature accounts of this action, videographic analysis revealed that male *C. funduloides* slap their pointed caudal peduncle tubercles against each other in maintaining their space. The tubercles may also serve as ornamentation for species and sex recognition, and threatening armament.

Microscopic Technique and Tubercle Development - Examination of the epidermis of these fishes by different methods of microscopy (LM and SEM) not only provided complete descriptions of cellular morphology and composition, but also of the gross morphology and distribution of surface features (e.g. tubercles). Application of a single microscopic technique is inadequate when both subepidermal and surface descriptions are required for epidermal characterization. For example, Roberts (1982) described the horny projections that arise from single cells in species of 15 ostariophysian families as unculi, and used the feature as a character to group all members in the superorder. In my examinations of some of the same fishes, structures resembling unculi were found when using SEM, but not with LM. The unculus-like structures (seen with SEM) were identified with LM as either nucleate squamate surface epidermal cells or neuromasts. Roberts (personal communication, 1992) upon learning that unculi are not horny (i.e., keratin not present), and are not present in histological sections, questioned his previous characterization of unculi. He suggested that nerve cells may act as organizers for epidermal structures.

In morphological studies of breeding tubercles in anal fin rays of the cyprinid *Oryzias latipes*, Egami and Nambu (1961) indicated that vital staining of tubercles on fin rays of males

with methylene blue showed better innervation than those of females which lacked tubercules (= contact organs). Similarily, Kaill (unpublished) cited in Foster (1967), who used spinal staining techniques, stated that each anal fin contact organ in the cyprinid *Nothobranchius guentheri* appear to receive a lateral branch from a nerve fiber which runs the length of the fin ray.

In my SEM examination of the snout region of sub-adult male *C. funduloides*, developing tubercles were spaced along the posterior portion of the infraorbital sensory canal. The anterior pores of the infraorbital sensory canal had neuromasts. In between the neuromasts and tubercles of the infraorbital canal were three sensory canal pores that appeared transitional, forming elevated mounds of cells like those observed in adult male specimens.

Dermal pilli underlie tubercles in the cyprinids in this study. Tubercles probably serve as tactile sensory receptors (= contact organs): two literature sources provide supporting evidence that individual anal fin tubercles receive a distinct nerve fiber (Kaill); and innervation is increased below tubercles (Egami and Nambu, 1961)

Breeding tubercles, as annual growths, occur in distinct species-specific patterns on fishes. Nerve staining techniques will be required to test the hypothesis that neurons underlying the epidermis serve as organizing templates for species-specific tubercle patterns that cyprinid males exhibit during the spawning season.

Literature Cited

Branson, B. A. 1962. Observations on the breeding tubercles of some Ozarkian minnows with notes on the barbels of *Hybopsis*. Copeia 1962(3):532-539.

Cason, J. E. 1950. A rapid one-step Mallory-Heidenhain stain for connective tissue. Stain Technology 25:225-226.

Denoncourt, R. F. 1969. Fishes that grow horns. The Conservationist (State of New York Conservation Association) 2:30-31.

Duebler, E. E. 1955. A taxonomic study of the cyprinid fish *Clinostomus vandoisulus* (Valenciennes) in the eastern United States. Ph.D. thesis, Cornell University 167 p.

Egami, N. and M. Nambu. 1961. Factors initiating mating behavior and oviposition in the fish, *Oryzias latipes*. Journal of Faculty of Science (University of Tokyo) sect. 49(2):263-278.

Foster, N. R. 1967. Trends in the reproductive behavior of killifishes. Studies in Tropical Oceanography 5:549-566.

Fowler, H. W. 1912. Some features of ornamentation in fresh water fishes. The American Naturalist 46(548):470-476.

Harris, J.E. and S. Hunt 1975. The fine structure of the epidermis of two species of salmonid fish, the Atlantic Salmon *(Salmo salar)* and the Brown Trout *(Salmo trutta)* I. General Organization And Filament Containing Cells. II. Mucous Cells. Cell Tissue Research 163:535-543.

Henrikson, R. C. and A. G. Maltoltsy. 1968a. The fine structure of teleost epidermis, introduction and filament containing cells. Journal of Ultrastructure Research

21:194-212.

_____ and _____. 1968b. The fine structure of teleost epidermis, mucous cells, Journal of Ultrastructure Research 21:213-221.

_____ and _____. 1968c. The fine structure of teleost epidermis, club cells and other cell types, Journal of Ultrastructure Research 21:222-232.

Hubbs, C. L. and B. Walker. 1942. Habitat and breeding behavior of the American cyprinid fish *Notropis longirostris*. Copeia 1942:101-104

Humason, G. L. 1967. *Animal Tissue Techniques*. W.H. Freeman and Co., San Francisco © 1967.

Koster, W. J. 1939. Some phases of the life history and relationships of the cyprinid, *Clinostomus elongatus*. Copeia 1939(4):201-208.

Maurakis, E. G. and W. S. Woolcott. 1989a. *Reproductive behavior of the creek chub Semotilus atromaculatus*. Video, RaukWool Productions, University of Richmond, VA. 23173.

_____, and _____. 1989b. *Reproductive Behavior of Campostoma anomalum*. Video, RaukWool Productions, University of Richmond 23173.

_____, _____, and J. T. Magee. 1990. Pebble nests of four *Semotilus* species. Southeastern Council of Fishes PROCEEDINGS 22:7-13.

_____, _____, and M. H. Sabaj. 1991a. Reproductive behavioral phylogenetics of *Nocomis* species-groups. American Midland Naturalist 126:103-110.

_____, _____, and _____. 1991b. Reproductive behavior of Exoglossum species. Bulletin of the Alabama Museum of

Natural History 102:11-16.

_____, _____, and _____. 1992. Water currents in spawning areas of pebble nests of *Nocomis leptocephalus* (Pisces: Cyprinidae). Southeastern Council of Fishes PROCEEDINGS (25):1-3.

Miller, R. J. 1962. Reproductive behavior of the stoneroller minnow, *Campostoma anomalum pullum.* Copeia 1962(2):407-417.

Pfeiffer, W. 1977. The distribution of fright reaction and alarm substance cells in fishes. Copeia 1977(4):653-665.

_____. 1978. Heterocyclic compounds as releasers of fright reaction in the giant danio *Danio malabaricus* (Jerdon) (Cyprinidae, Ostariophysi, Pisces). Journal of Chemical Ecology 4(6): 665-673.

Raney, E.C. 1940. The breeding behavior of the common shiner, *Notropis cornutus* (Mitchill). Zoologica 25: 1-14.

Reed, J. R.,W. Weiland, and T. D. Kimbrough. 1972. A study on the biochemistry of alarm substances in fish. 26th Annual Conference of Southeastern Association of Game and Fish Commissioners PROCEEDINGS:608-610.

Reid, L. and J. R. Clamp. 1978. The biochemical and histochemical nomenclature of mucus. British Medical Bulletin 34(1):5-8.

Reighard, J. 1903. The function of pearl organs of the Cyprinidae. Science (new ser.) 17:531.

_____. 1910. Methods for studying the habits of fishes, with an account of the breeding habits of the horned dace. Bull. of U.S. Bureau of Fish 28(1908):1113-1136.

_____. 1920. The breeding behavior of the suckers and

minnows. Biological Bulletin (Woods Hole) 38(1):1-32.

Roberts, T. R. 1982. Unculi (horny projections arising from single cells) an adaptative feature of the epidermis of ostatiophysan fishes. Zoologica Scripta, 11(1):55-76.

Sabaj, M. H. 1992. *Spawning Clasps and Gamete Deposition in Pebble Nest-Building Minnows.* unpublished M.S. Thesis University of Richmond, Richmond, Virginia.

SAS Institute, Inc. 1985. *SAS User's Guide: Statistics. Version 5.* SAS Institute, INC. Cary, North Carolina.

Steele, R. G. and J. H. Torrie. 1980. *Principles and Procedures of Statistics.* McGraw-Hill Book Company, Inc. New York, New York.

Whitear, M. 1970. The skin surface of bony fishes. Journal of Zoology, London 160:437-454.

_____. 1984. The skin of fishes including cyclostomes, Chapter 2 Epidermis, Chapter 3 Dermis. In: *Biology of The Integument, Vertebrates.* Vol. 2 (J. Bereiter-Hahn, A.G. Maltoltsy, and K.S.Richards, eds), pp.9-64 Springer-Verlag;Berlin ©1986.

Wiley, M. L. and B. B. Collette. 1970. Breeding tubercles and contact organs in fishes: their occurrence, structure, and significance. Bulletin of the American Museum of Natural History 143(3):143-216.

Woolcott, W. S. and E. G. Maurakis. 1988. Pit ridge nest construction and spawning behaviors of *Semotilus lumbee* and *Semotilus thoreauianus*. Southeastern Council of Fishes PROCEEDINGS (18):1-3.

Table 1. **Snout region** of spawning male cyprinid nest-builders (*Nocomis leptocephalus, Semotilus atromaculatus,* and *Exoglossum maxillingua*), and male cyprinid non nest-builders (*Campostoma anomalum* and *Clinostomus funduloides*). N equals number of observations per region on a single specimen of each species (unicellular gland types: 1 = present: 0 = absent).

Character	*leptocephalus*	*atromaculatus*	*maxillingua*	*anomalum*	*funduloides*
Epidermal thickness					
mm(df 10, F 6.4)	0.34±.06	0.16±.01	0.11±.03	0.28±.11	0.16±.06
n	7	6	5	6	4
cells(df 29,F 69.5)	15	12	11	29	18
n	10	6	7	5	6
Tubercle keratin thickness					
mm(df 9, F 234.6)	0.44	1.04	-----	n/a	0.02
n	5	3	-----	-----	6
cells(df 11, F 13.6)	64	104	-----	n/a	4.8
n	4	4	-----	-----	5
Maximum tubercle height					
mm	2.09	0.88	-----	1.66	0.27
Sub-cuticle keratin depth					
mm	0.15	0.35	-----	1.14	0.04
n	5	4	-----	6	5
Unicellular gland types					
sacciform	1	0	1	0	0
club	0	0	1	0	0
mucous	1	0	0	0	0
serous	1	1	1	0	1

Table 2. Cheek region of spawning male cyprinid nest-builders (*Nocomis leptocephalus, Semotilus atromaculatus,* and *Exoglossum maxillingua*), and male cyprinid non nest-builders (*Campostoma anomalum* and *Clinostomus funduloides*). N equals number of observations per region on a single specimen of each species (unicellular gland types: 1 = present: 0 = absent).

Character	*leptocephalus*	*atromaculatus*	*maxillingua*	*anomalum*	*funduloides*
Epidermal thickness					
mm(df 10, F 7.2)	0.26±.02	0.11±.01	0.15±.05	0.28±.11	0.15±.04
n	6	3	4	4	5
cells(df 25, F 49.1)	20	10	11	29	18
n	16	6	5	5	7
Tubercle keratin thickness					
mm	-----	-----	-----	-----	0.03
n	-----	-----	-----	-----	6
cells	-----	-----	-----	-----	8
n	-----	-----	-----	-----	3
Maximum tubercle height					
mm	-----	-----	-----	-----	0.27
Sub-cuticle keratin depth					
mm	-----	-----	-----	-----	0.03
n	-----	-----	-----	-----	5
Unicellular gland types					
sacciform	1	0	1	0	0
club	0	1	1	0	0
mucous	0	0	0	0	0
serous	1	0	1	0	0

Table 3. **Mandible region** of spawning male cyprinid nest-builders (*Nocomis leptocephalus*, *Semotilus atromaculatus*, and *Exoglossum maxillingua*), and male cyprinid non nest-builders (*Campostoma anomalum* and *Clinostomus funduloides*). N equals number of observations per region on a single specimen of each species (unicellular gland types: 1 = present: 0 = absent).

Character	*leptocephalus*	*atromaculatus*	*maxillingua*	*anomalum*	*funduloides*
Epidermal thickness					
mm(df 10, F 13.3)	0.60±.15	0.31±.13	0.19±.04	0.11±.03	0.10±.02
n	7	6	4	5	7
cells(df 24, F 75.1)	60	16	14	21	8
n	3	8	6	6	7
Keratin					
internal surface	present	present	present	-----	-----
external surface	-----	-----	-----	present	-----
tuberculate	-----	-----	-----	-----	-----
Unicellular Gland Types					
sacciform	0	0	0	0	0
club	0	0	0	0	0
mucous	0	0	0	0	0
serous	0	0	0	0	0

Table 4: Pectoral fin region of spawning male cyprinid nest-builders (*Nocomis leptocephalus, Semotilus atromaculatus,* and *Exoglossum maxillingua*), and male cyprinid non nest-builders (*Campostoma anomalum* and *Clinostomus funduloides*). N equals number of observations per region on a single specimen of each species (unicellular gland types: 1 = present: 0 = absent).

Character	*leptocephalus*	*atromaculatus*	*maxillingua*	*anomalum*	*funduloides*
Epidermal thickness					
mm(df 10, F 99.1)	0.09±.02	0.02±.01	0.04±.01	0.08±.04	0.06±.05
n	3	3	3	3	3
cells(df 33, F 11.9)	13	7	8	14	7
n	8	9	7	5	6
Tubercle keratin thickness					
mm(df 17, F 3.19)	0.05	0.04	-----	n/a	0.03
n	3	9	-----	-----	6
cells(df 14, F 12.2)	9	6	-----	n/a	7
n	7	3	-----	-----	5
Maximum tubercle height					
mm	1.03	0.25	-----	0.35	0.24
Sub-cuticle keratin depth					
mm	0.08	0.00	-----	0.27	0.00
n	4	n/a	-----	6	n/a
Unicellular Gland Types					
sacciform	0	0	1	0	0
club	1	0	1	1	0
mucous	1	0	0	0	0
serous	1	1	1	0	1

Table 5. **Anterior dorso-lateral trunk region** of spawning male cyprinid nest-builders (*Nocomis leptocephalus, Semotilus atromaculatus,* and *Exoglossum maxillingua*), and male cyprinid non nest-builders (*Campostoma anomalum* and *Clinostomus funduloides*). N equals number of observations per region on a single specimen of each species (unicellular gland types: 1 = present: 0 = absent).

Character	*leptocephalus*	*atromaculatus*	*maxillingua*	*anomalum*	*funduloides*
Epidermal thickness					
mm(df 10, F 1.9)	0.08±.04	0.07±.03	0.04±.01	0.34±.34	0.04±.01
n	4	4	3	10	3
cells(df 24, F 26.0)	22	13	5	26	6
n	10	7	6	9	8
Tubercle keratin thickness					
mm	-----	-----	-----	n/a	0.01
n	-----	-----	-----	-----	10
cells	-----	-----	-----	n/a	4
n	-----	-----	-----	-----	5
Maximum tubercle height					
mm	-----	-----	-----	0.75	0.11
Sub-cuticle keratin depth					
mm	-----	-----	-----	0.31	0.02
n	-----	-----	-----	3	4
Unicellular gland types					
sacciform	1	0	0	0	0
club	1	0	1	0	1
mucous	0	0	0	0	0
serous	1	1	1	0	1

Table 6. Caudal peduncle region of spawning male cyprinid nest-builders (*Nocomis leptocephalus, Semotilus atromaculatus,* and *Exoglossum maxillingua*), and male cyprinid non nest-builders (*Campostoma anomalum* and *Clinostomus funduloides*). N equals number of observations per region on a single specimen of each species (unicellular gland types: 1 = present: 0 = absent).

Character	*leptocephalus*	*atromaculatus*	*maxillingua*	*anomalum*	*funduloides*
Epidermal thickness					
mm(df 10, F 5.1)	0.08±.02	0.03±.02	0.03±.01	0.40±.27	0.02±.01
n	3	4	5	7	4
cells(df 24, F 11.2)	10	12	6	17	6
n	5	8	8	3	6
Tubercle keratin thickness					
mm(df 6, F 65.4)	-----	0.04	-----	n/a	0.01
n	-----	3	-----	-----	5
cells(df 9, F 61.9)	-----	11	-----	n/a	3
n	-----	4	-----	-----	7
Maximum tubercle height					
mm	-----	0.14	-----	0.40	0.07
Sub-cuticle keratin depth					
mm	-----	0.00	-----	0.38	0.00
n	-----	n/a	-----	2	n/a
Unicellular gland types					
sacciform	0	0	0	0	0
club	1	0	1	0	0
mucous	0	0	0	0	0
serous	1	1	1	0	1

Table 7. Summary of epidermal morphology of cyprinid male pebble nest-builders (*Nocomis leptocephalus*, *Semotilus atromaculatus*, and *Exoglossum maxillingua*); and male cyprinid non nest-builders (*Campostoma anomalum* and *Clinostomus funduloides*).

Region	Species				
	leptocephalus	*atromaculatus*	*maxillingua*	*anomalum*	*funduloides*
Snout					
Tubercles	present	present	absent	present	present
Mandible					
Keratinization					
internal	present	present	present	absent	absent
external	absent	absent	absent	present	present
Tubercles	absent	absent	absent	absent	present
Collagenous dermal anchorage	absent	absent	absent	present	absent
Cheek					
Specialized cell types	absent	keratin	absent	absent	keratin
Tubercles	absent	absent	absent	absent	present
Pectoral fin					
Ray tubercles	present	present	absent	present	present
Body					
Trunk tubercles	absent	absent	absent	centered	marginal
Type	--	--	--	pointed	pointed
Caudal peduncle tubercles	absent	marginal	absent	centered	marginal
Type	--	smooth	--	pointed	pointed

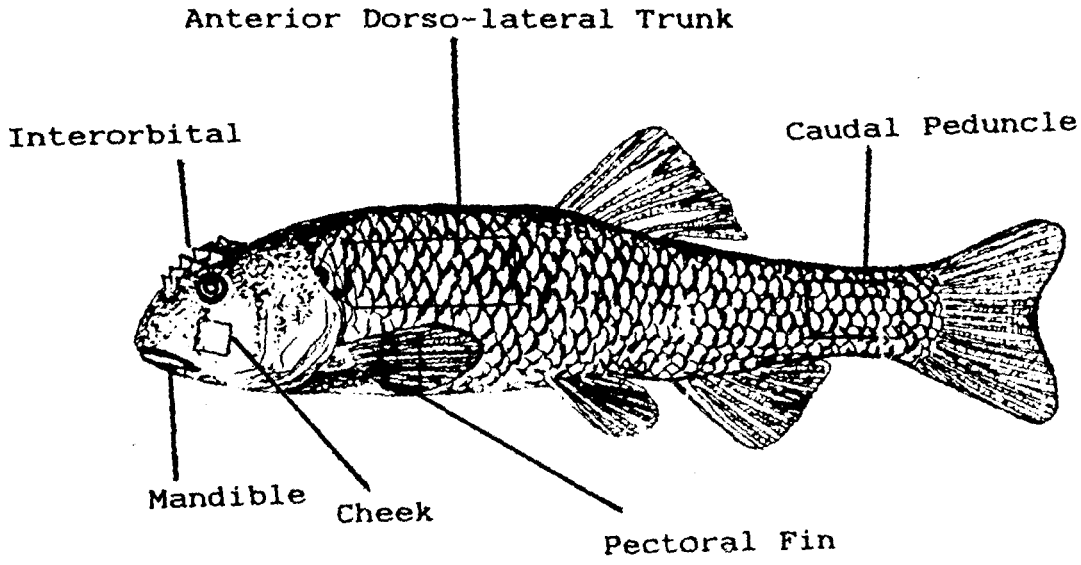
Table 8. Summary of epidermal function to breeding behaviors in male cyprinid pebble nest-builders (*Nocomis leptocephalus*, *Semotilus atromaculatus*, and *Exoglossum maxillingua*) and male cyprinid non nest-builders (*Campostoma anomalum* and *Clinostomus funduloides*).

Region	Species				
	leptocephalus	***atromaculatus***	***maxillingua***	***anomalum***	***funduloides***
Snout	Combat	Same	--	Same	Same
	Ornamentation	Same		Same	Same
Mandible	Nest Construction	Same	Same	Excavation	Combat
					Ornamentation
Cheek	--	Nest Construction	--	--	Combat
					Ornamentation
Pectoral fin	Spawning	Same	--	Same	Same?
Body Trunk	Spawning[a]	Same	Same	Combat	Same
Caudal	Spawning[a]	Same	Same	Combat	Same

[a] - keratin absent

Figure 1.

Drawing of fish body regions.


Anterior Dorso-lateral Trunk
Interorbital
Caudal Peduncle
Mandible
Cheek
Pectoral Fin

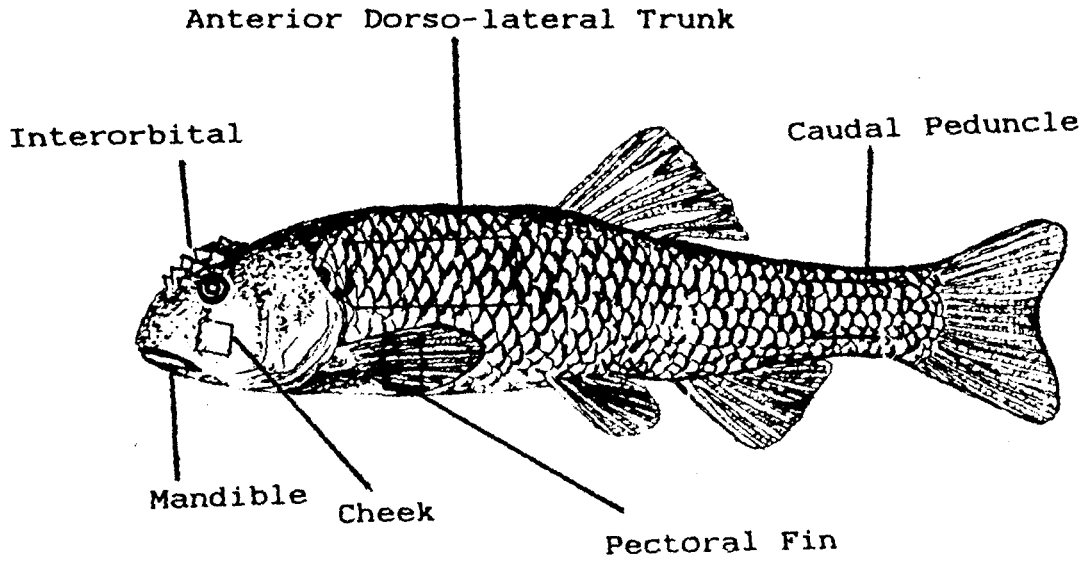

Anterior Dorso-lateral Trunk
Interorbital
Caudal Peduncle
Mandible
Cheek
Pectoral Fin

Figure 2.

Drawing of epidermal cell types, tubercle measurements, and dermal pilli.

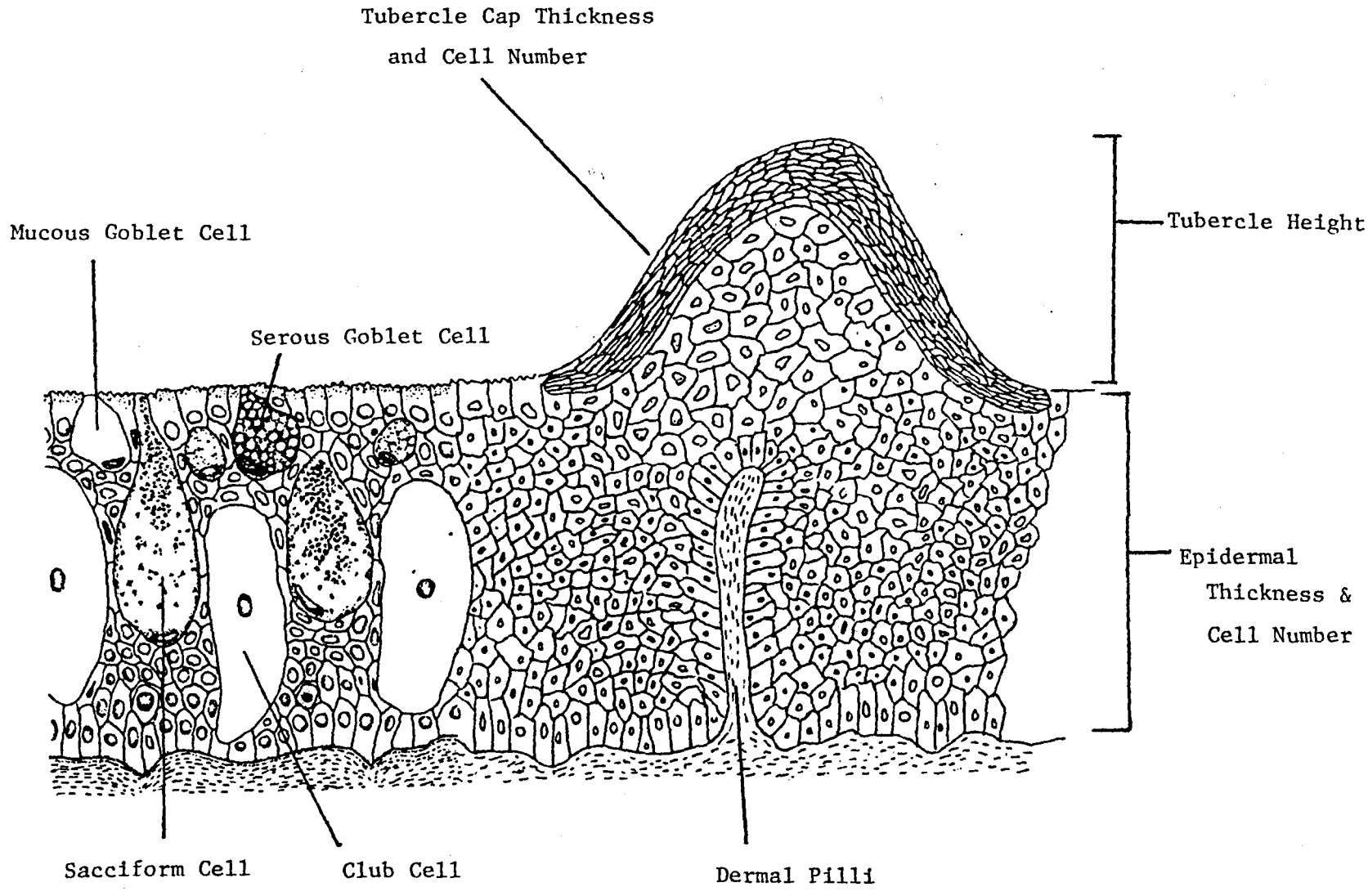

Tubercle Cap Thickness
and Cell Number
Mucous Goblet Cell
Serous Goblet Cell
Tubercle Height
Epidermal
Thickness &
Cell Number
Sacciform Cell
Club Cell
Dermal Pilli

Figure 3.

Scanning electron micrograph of worn keratin cells on a tubercle in snout region of *Nocomis leptocephalus* (x2800).

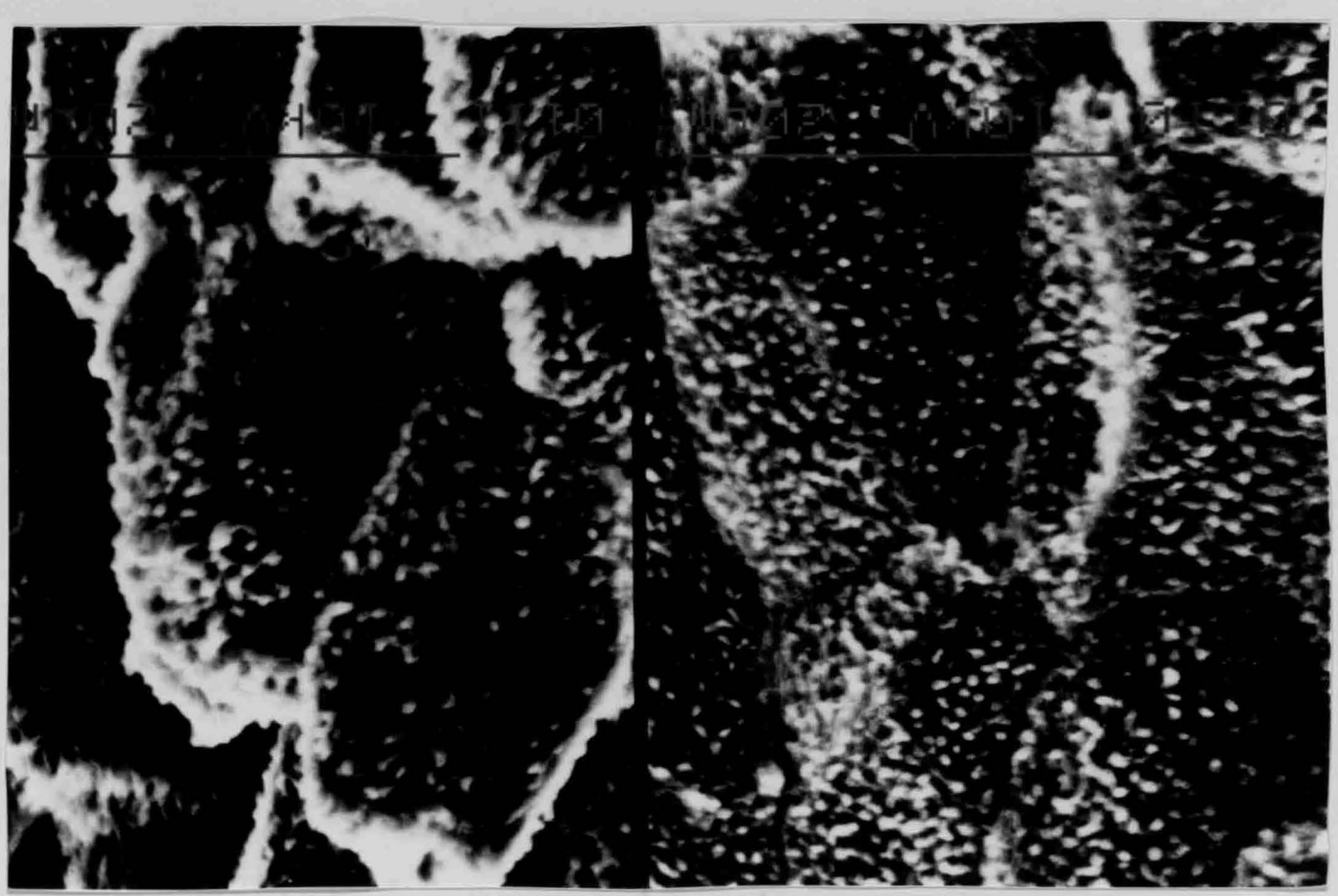


Figure 4.

Scanning electron micrograph of wide based, short keratin tubercles on snout region of *Semotilus atromaculatus* (x367).

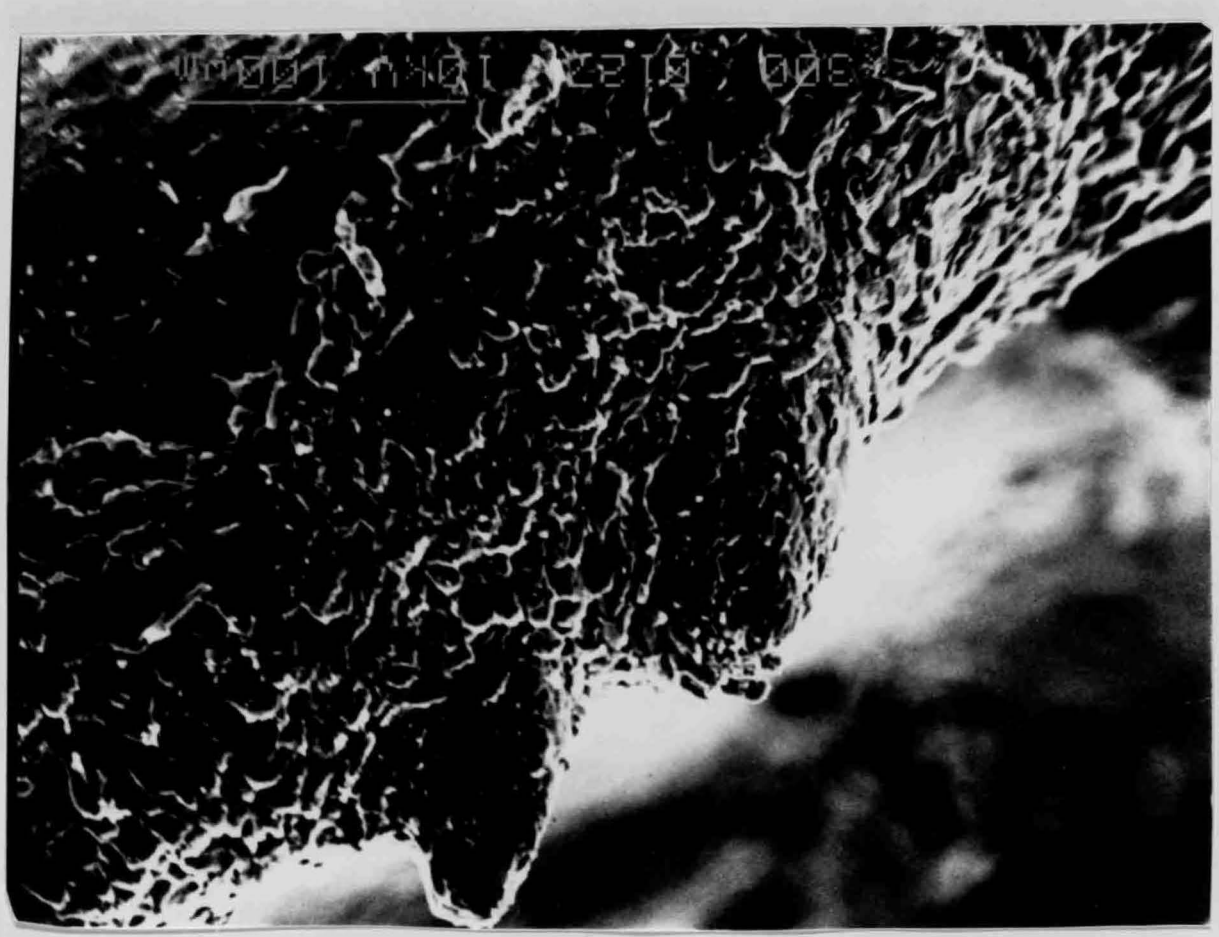


Figure 5.

Scanning electron micrograph of small conical tubercles on snout region of *Clinostomus funduloides* (x248).

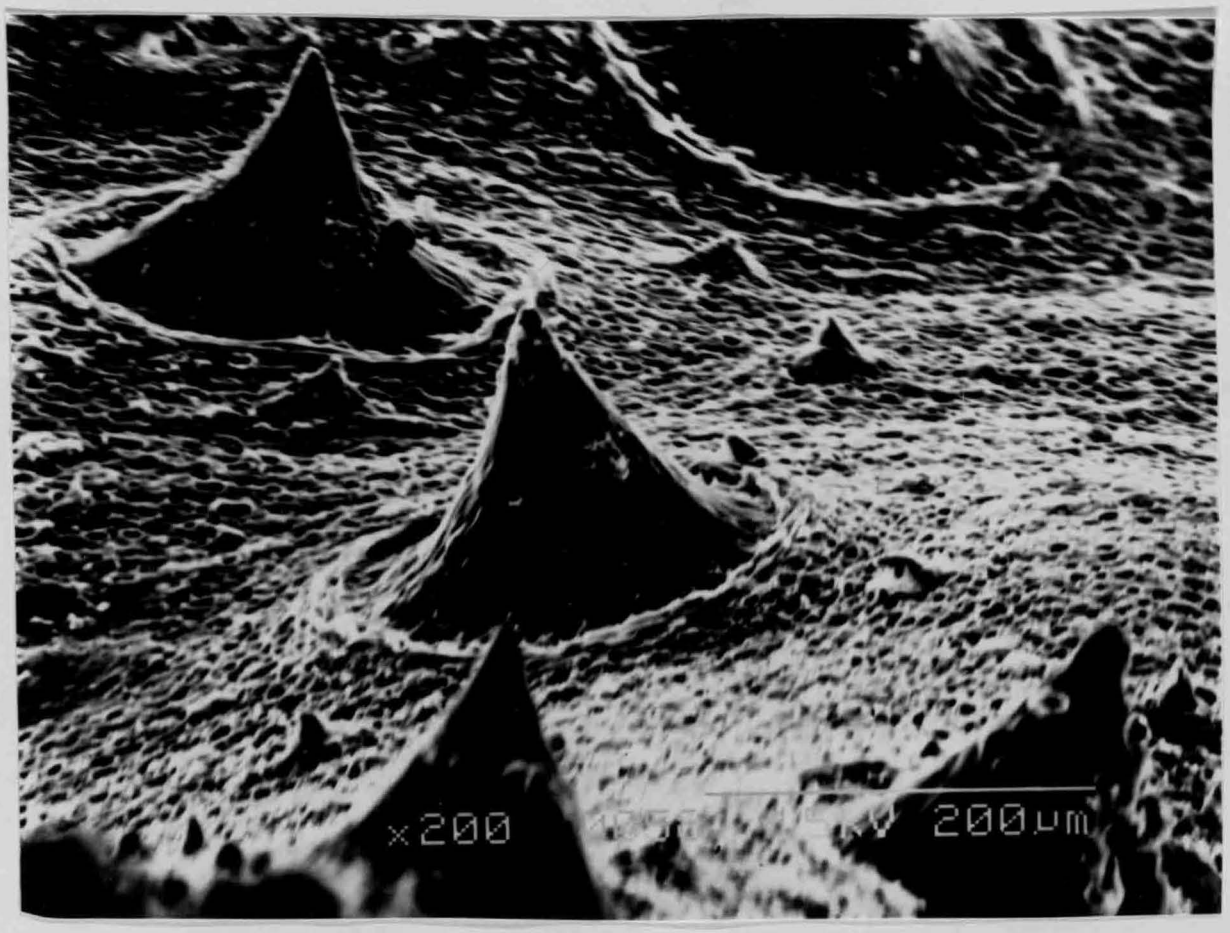

x200
200um

Figure 6.

Light micrograph of surface keratinized cells (K) in snout region of *Semotilus atromaculatus* (hematoxylin and eosin, x1243).

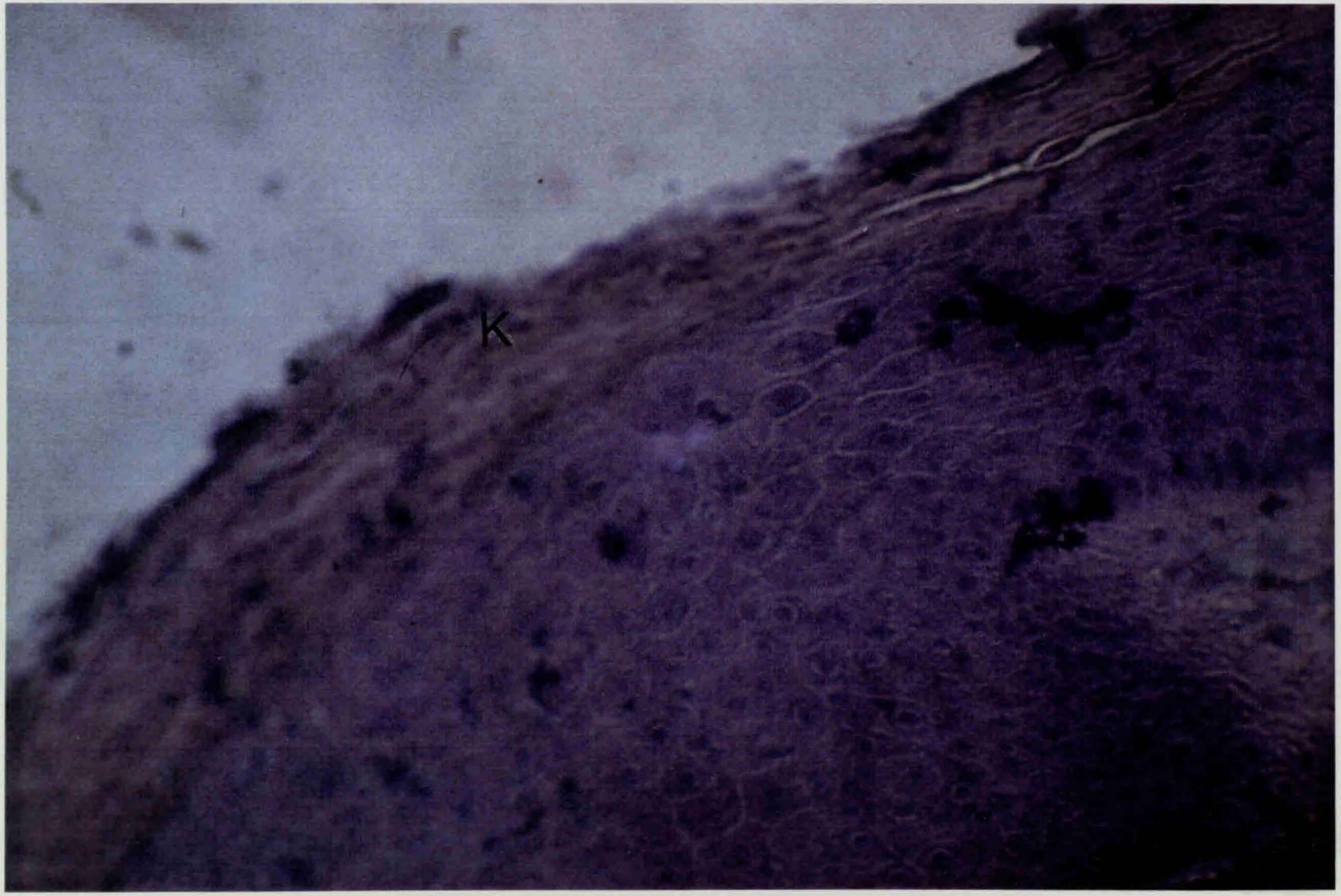

K

Figure 7.

Light micrograph of deeply-rooted tubercle enveloping epidermis (E) on snout region of *Campostoma anomalum* (Mallory's Heidenhain, x597).

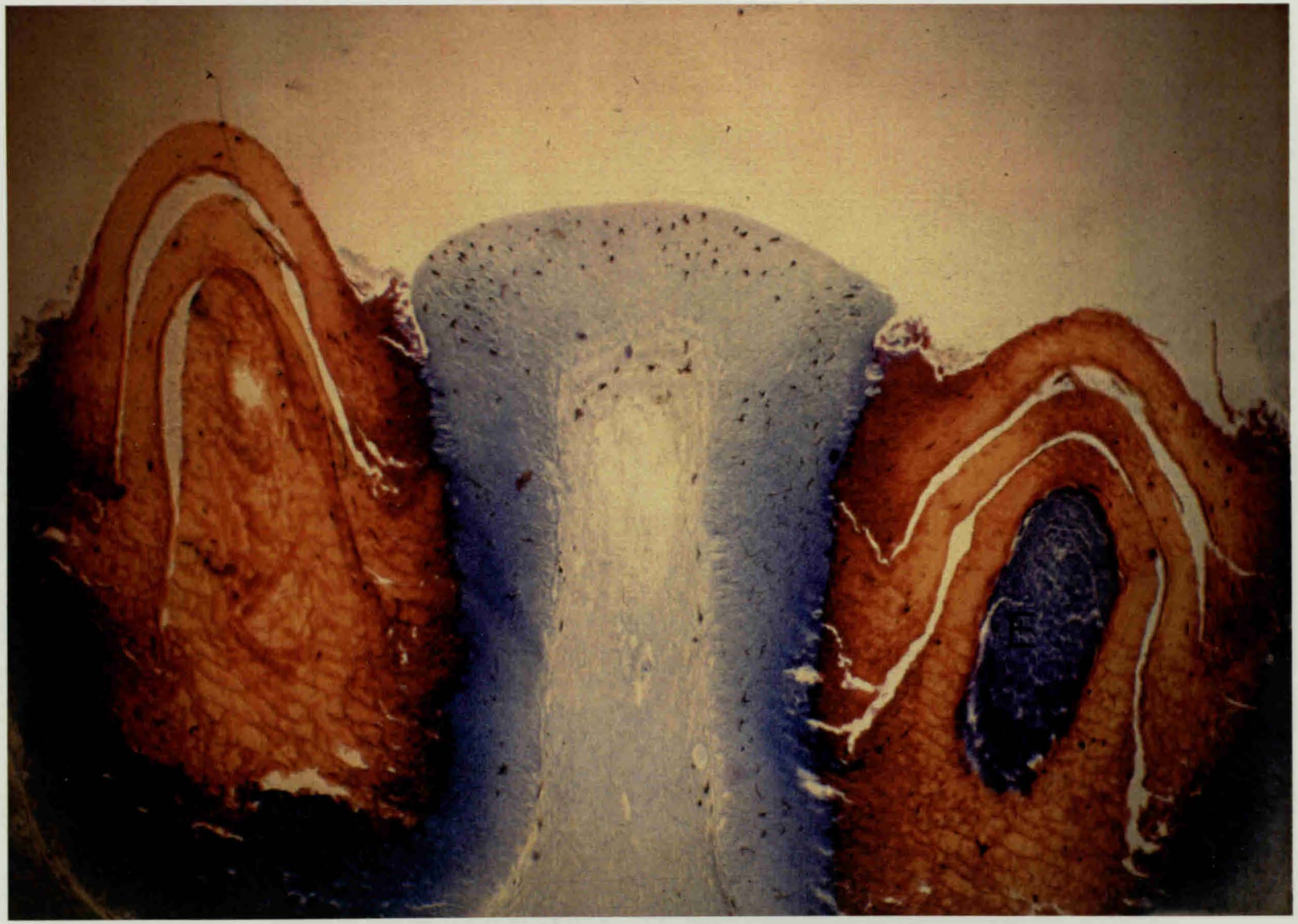


Figure 8.

Scanning electron micrograph of neuromast on snout region of *Exoglossum maxillingua* (x3750).

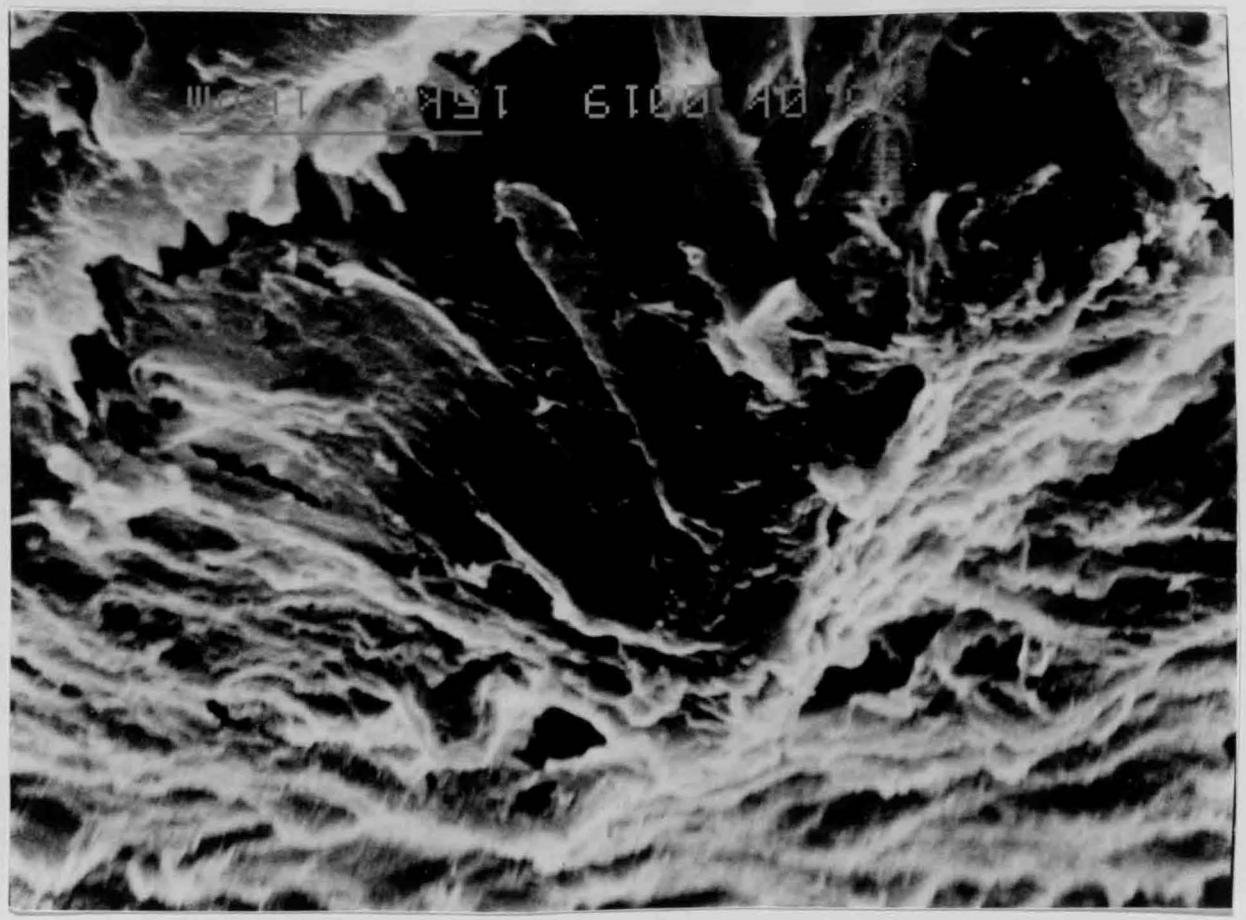


Figure 9.

Light micrograph of mitotic stages of cells below the developing tubercles in the cheek region of *Clinostomus funduloides* (Mallory's Heidenhain, x6257).

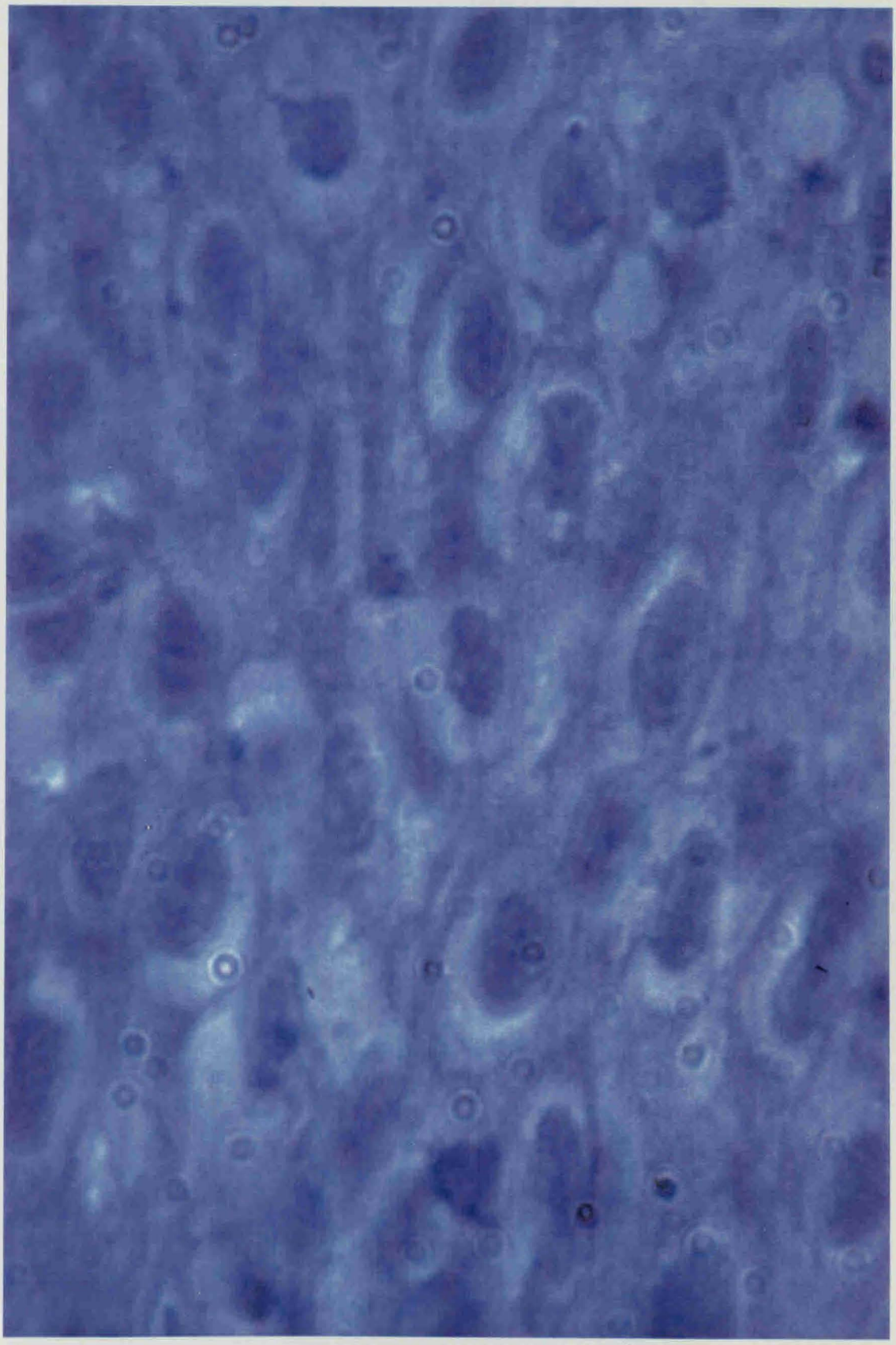


Figure 10.

Birefringent micrograph of cheek epidermis with cuticle (C) of *Campostoma anomalum* (hematoxylin and eosin, x664).

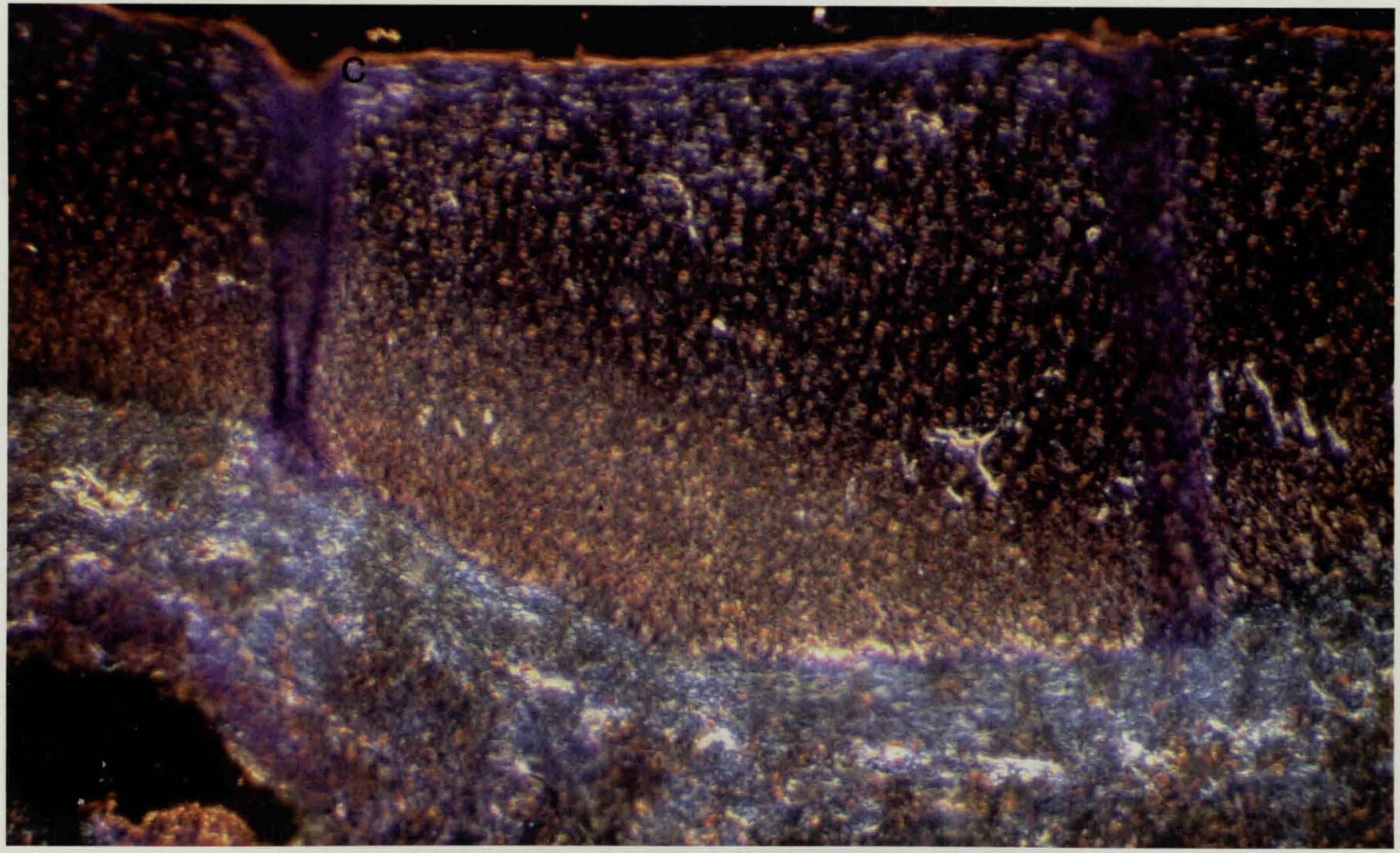

C

Figure 11.

Scanning electron micrograph of mandible tubercles of *Clinostomus funduloides* (x180).

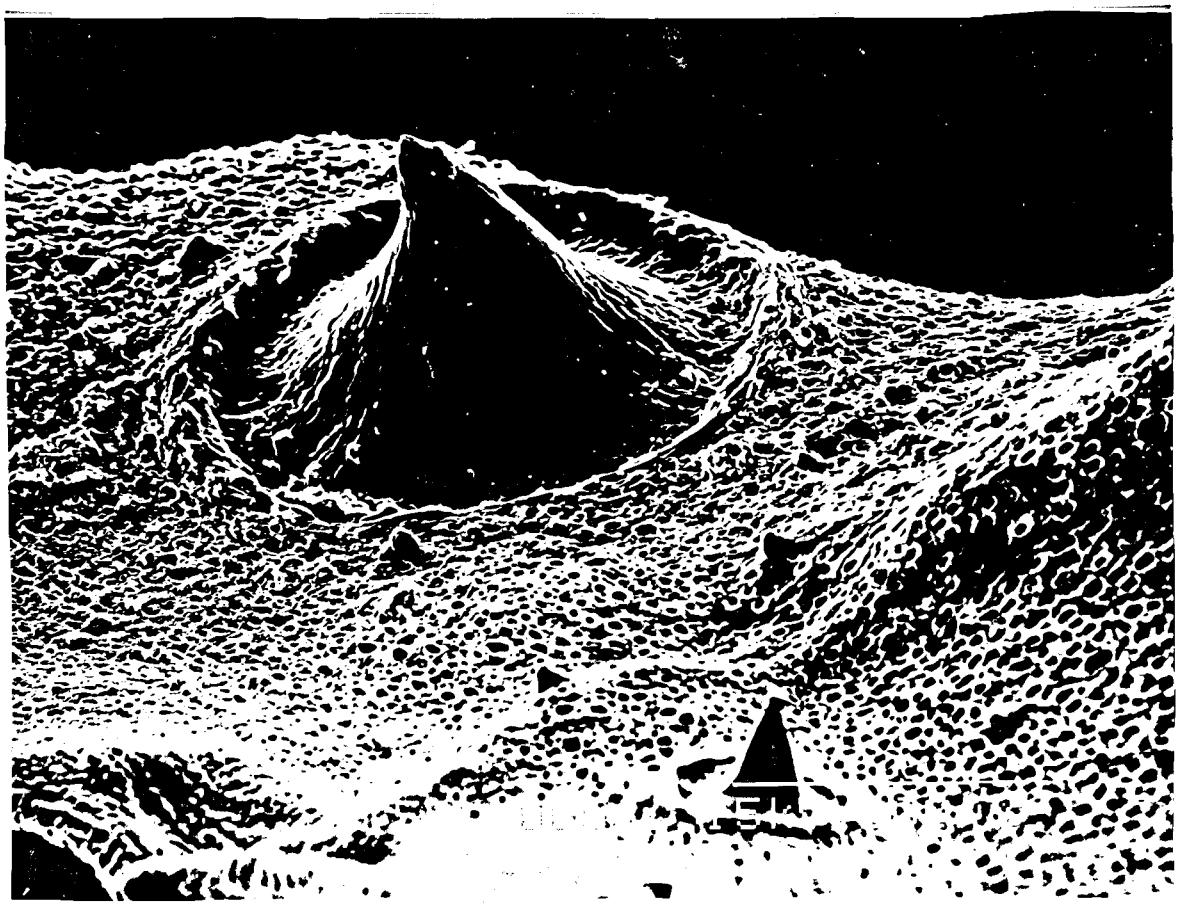


Figure 12.

Light micrograph of plugs of keratinized cells (stained red) in mandibular epidermis of *Semotilus atromaculatus* (Mallory's Heidenhain, x622).

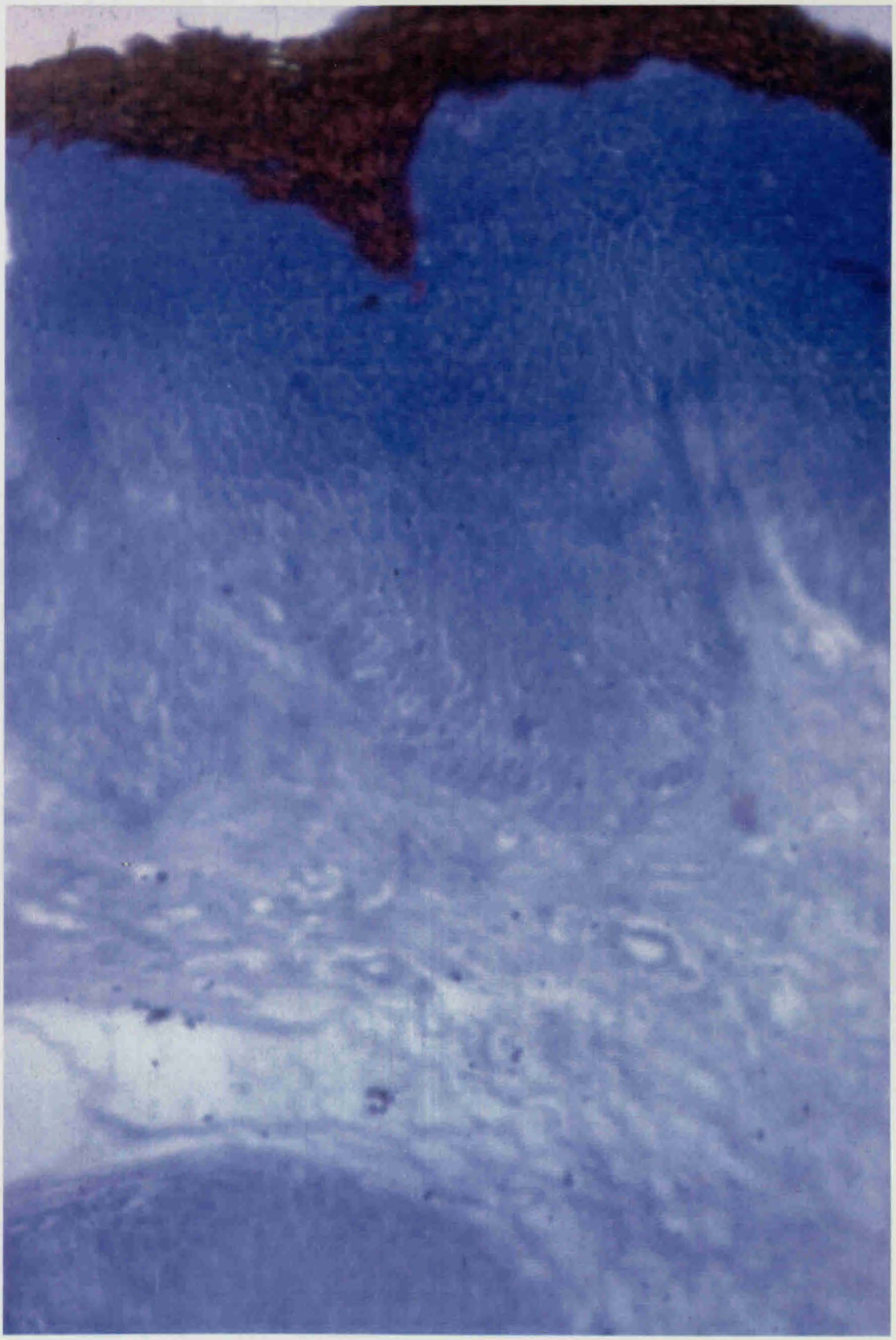


Figure 13.

Light micrograph of collagenous dermal pilli (DP) into interior epidermis in the mandible region of *Nocomis leptocephalus* (hematoxylin and eosin, x1251).

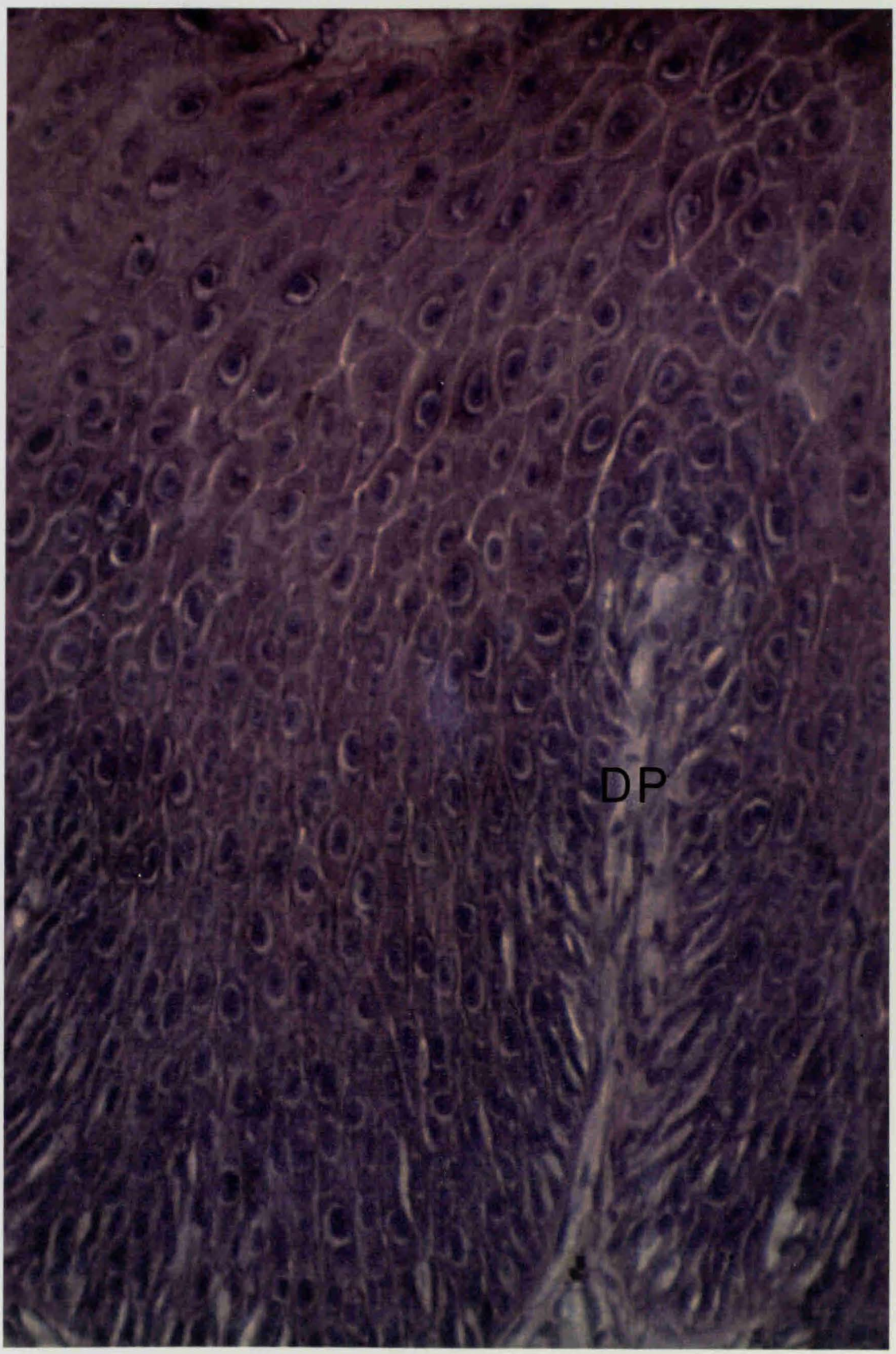

DP

Figure 14.

Light micrograph of long dermal strands (DS) and keratinized exterior surface of epidermis (E) on mandible region of *Campostoma anomalum* (Mallory's Heidenhain, x622).

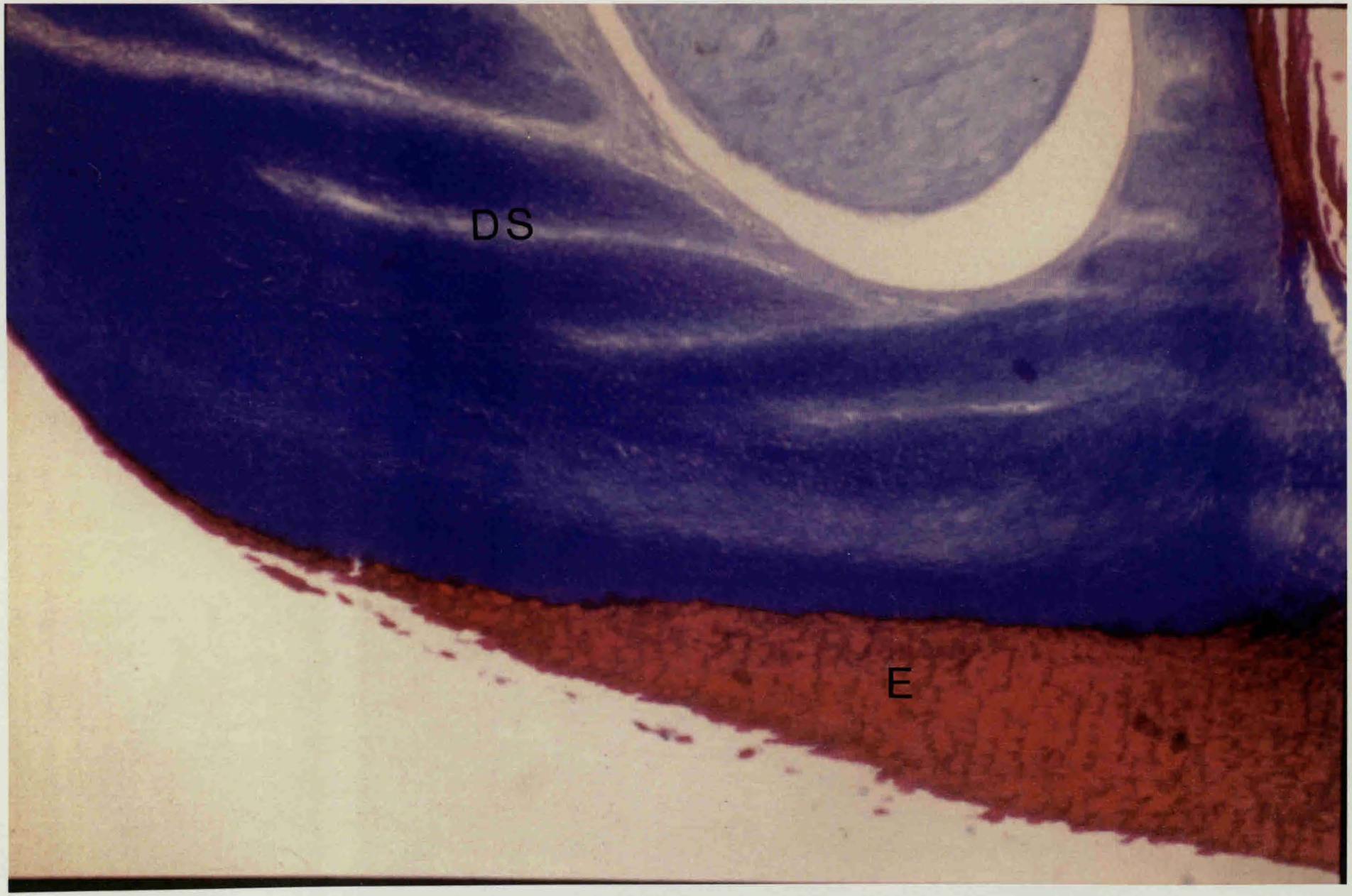

DS
E

Figure 15.

Light micrograph of keratin cells (K) on the inner surface of the mandible in *Exoglossum maxillingua* (Mallory's Heidenhain, x1251).

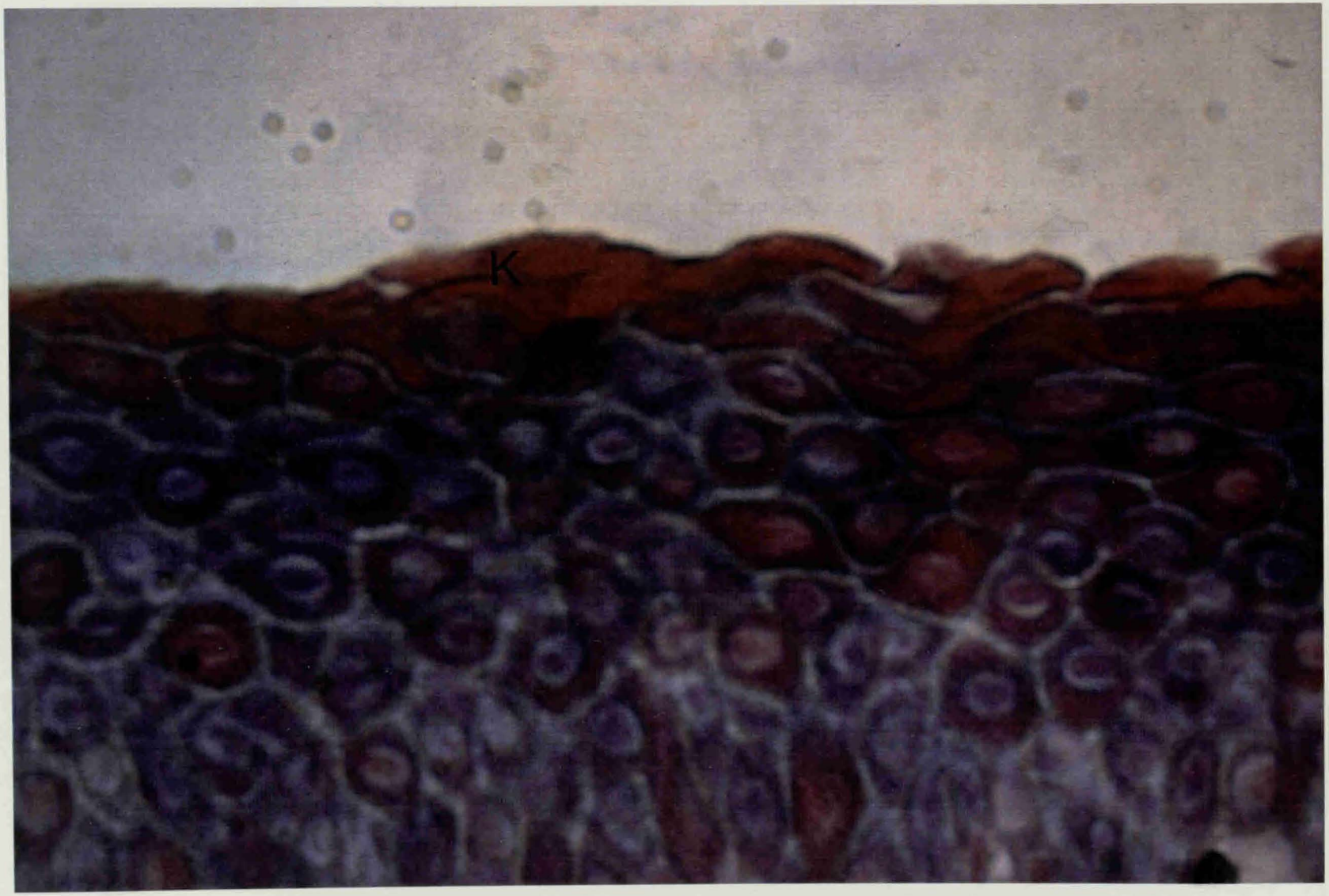

K

Figure 16.

Scanning electron micrograph of recurved pectoral fin tubercle on *Nocomis leptocephalus* (x184).

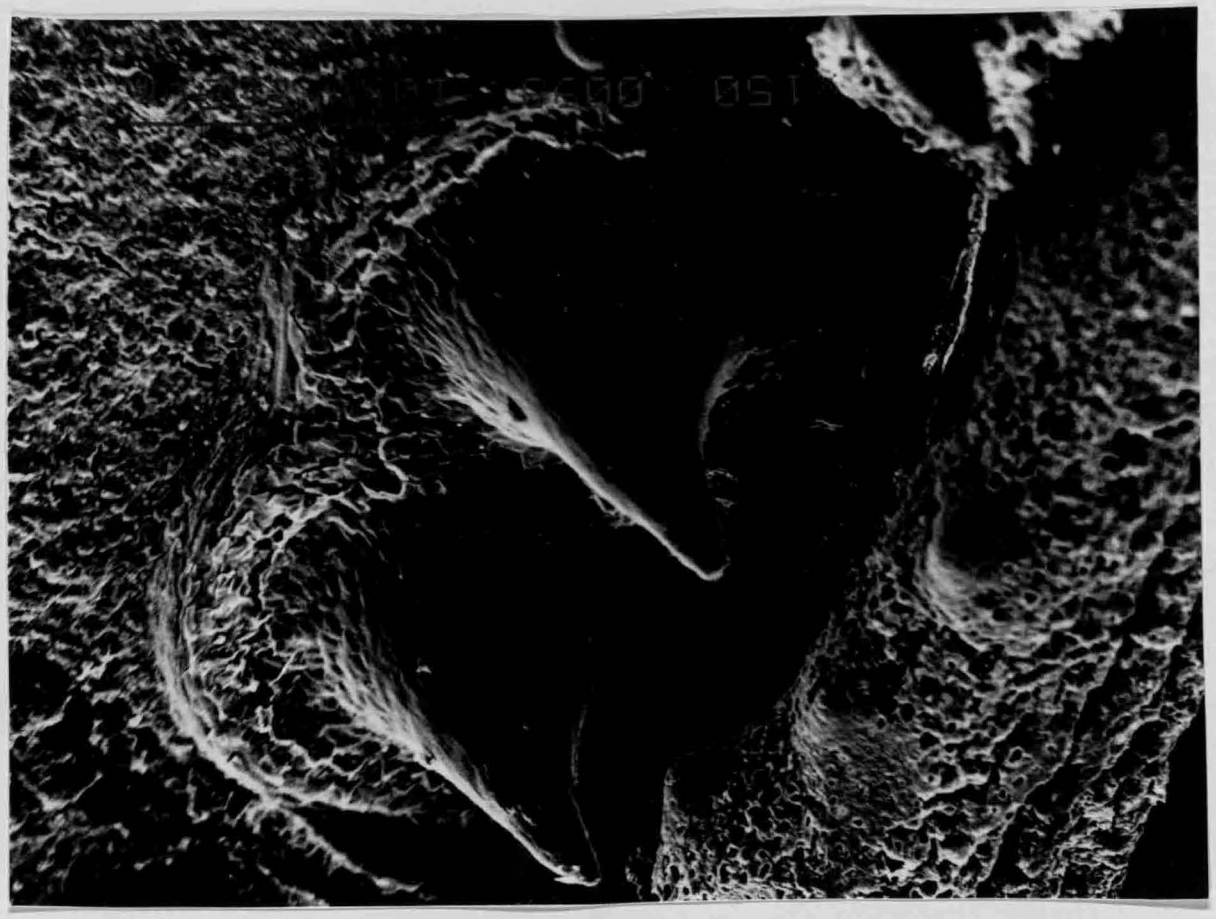


Figure 17.

Light micrograph showing lack of supportive dermis (D) between tubercle (T) and pectoral fin ray (F) in *Campostoma anomalum* (hematoxylin and eosin, x348).

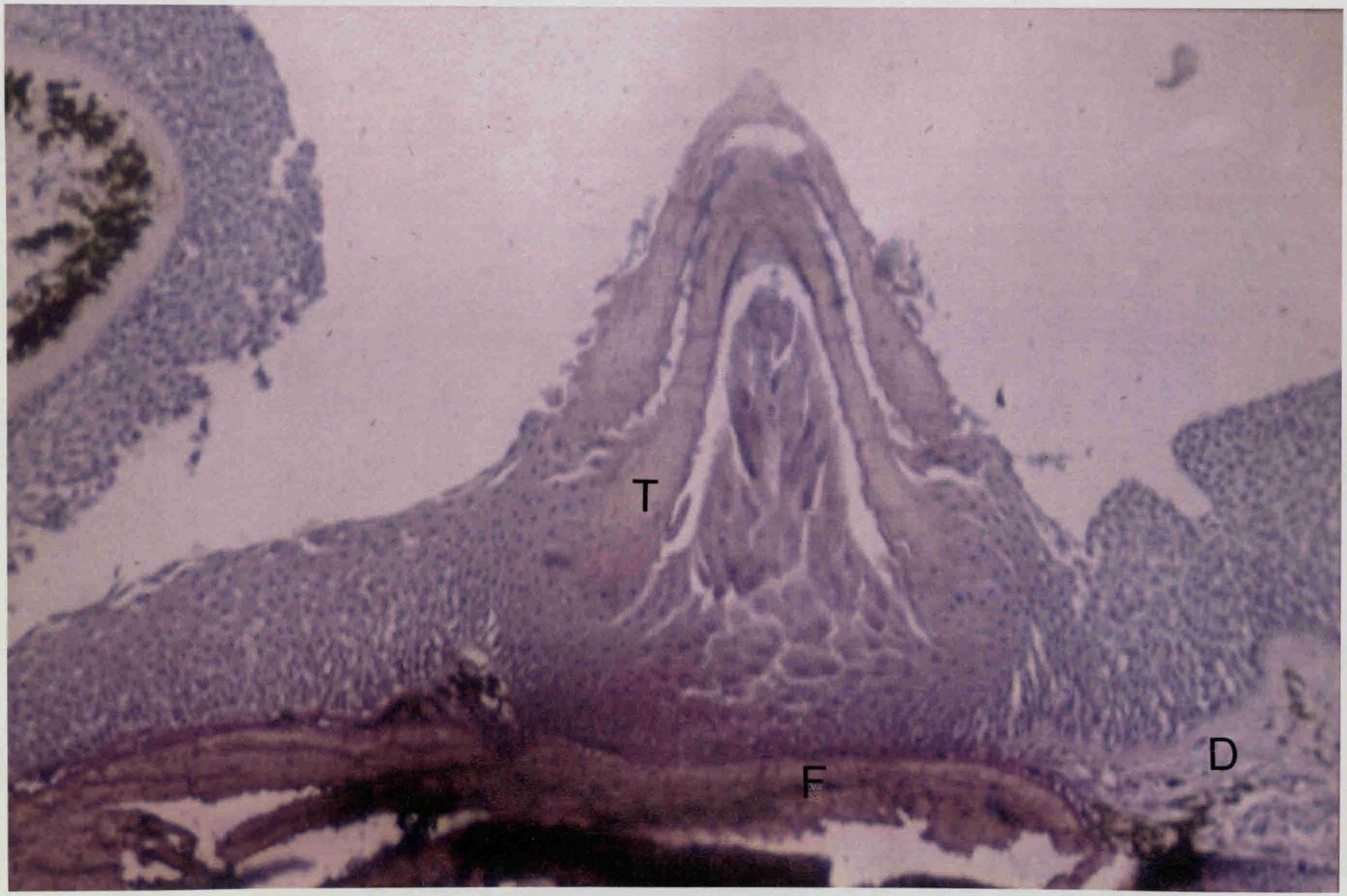

T
F
D

Figure 18.

Scanning electron micrograph of centered anterior dorso-lateral trunk tubercles on scales of *Campostoma anomalum* (x146).

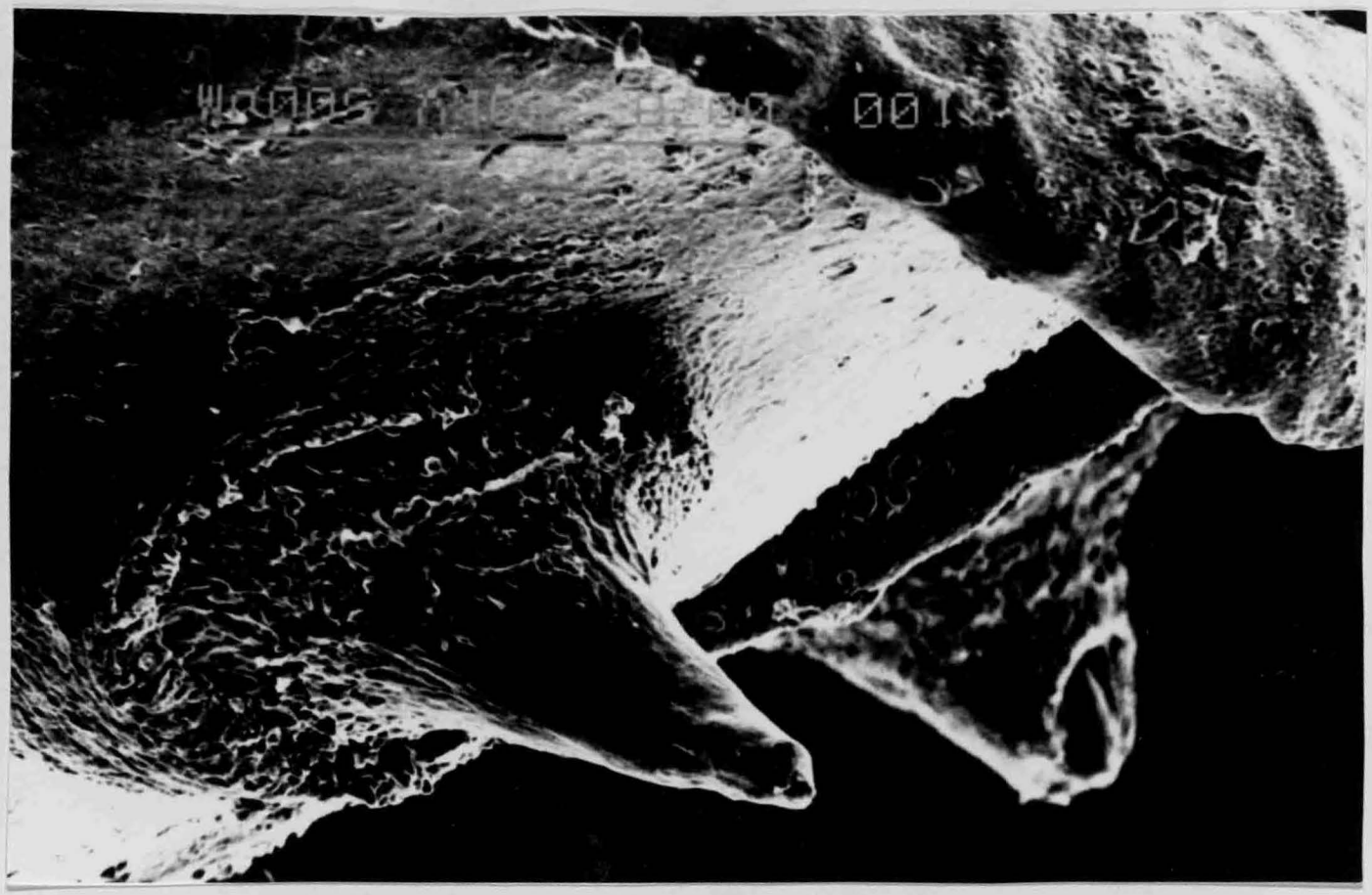


Figure 19.

Scanning electron micrograph outlining tubercle on posterior margin of anterior dorso-lateral trunk of *Clinostomus funduloides* (x576).

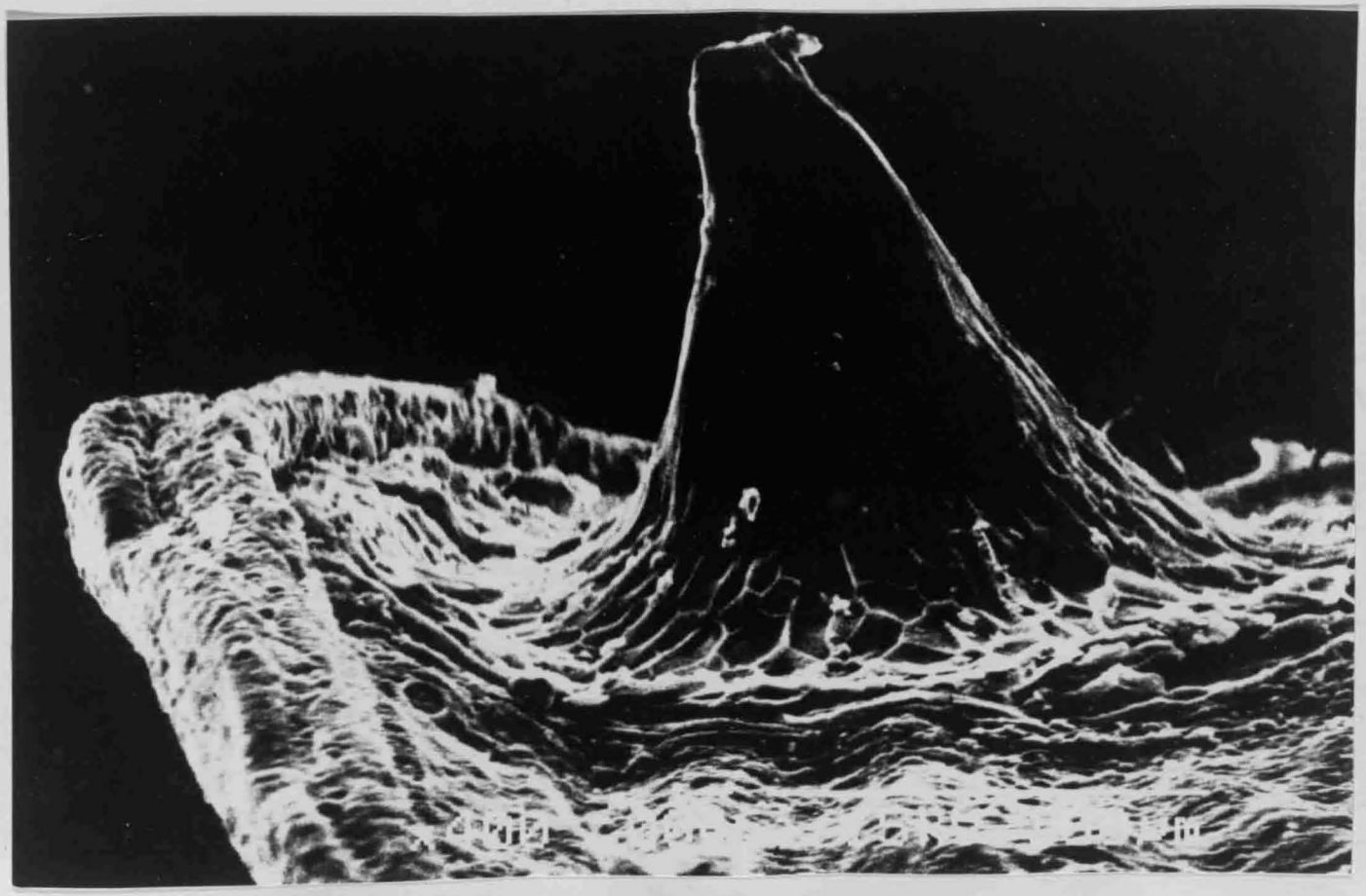


Figure 20.

Light micrograph of deeply rooted tubercles and dermal pilli (DP) on anterior dorso-lateral trunk scales of *Campostoma anomalum* (Mallory's Heidenhain, x662).

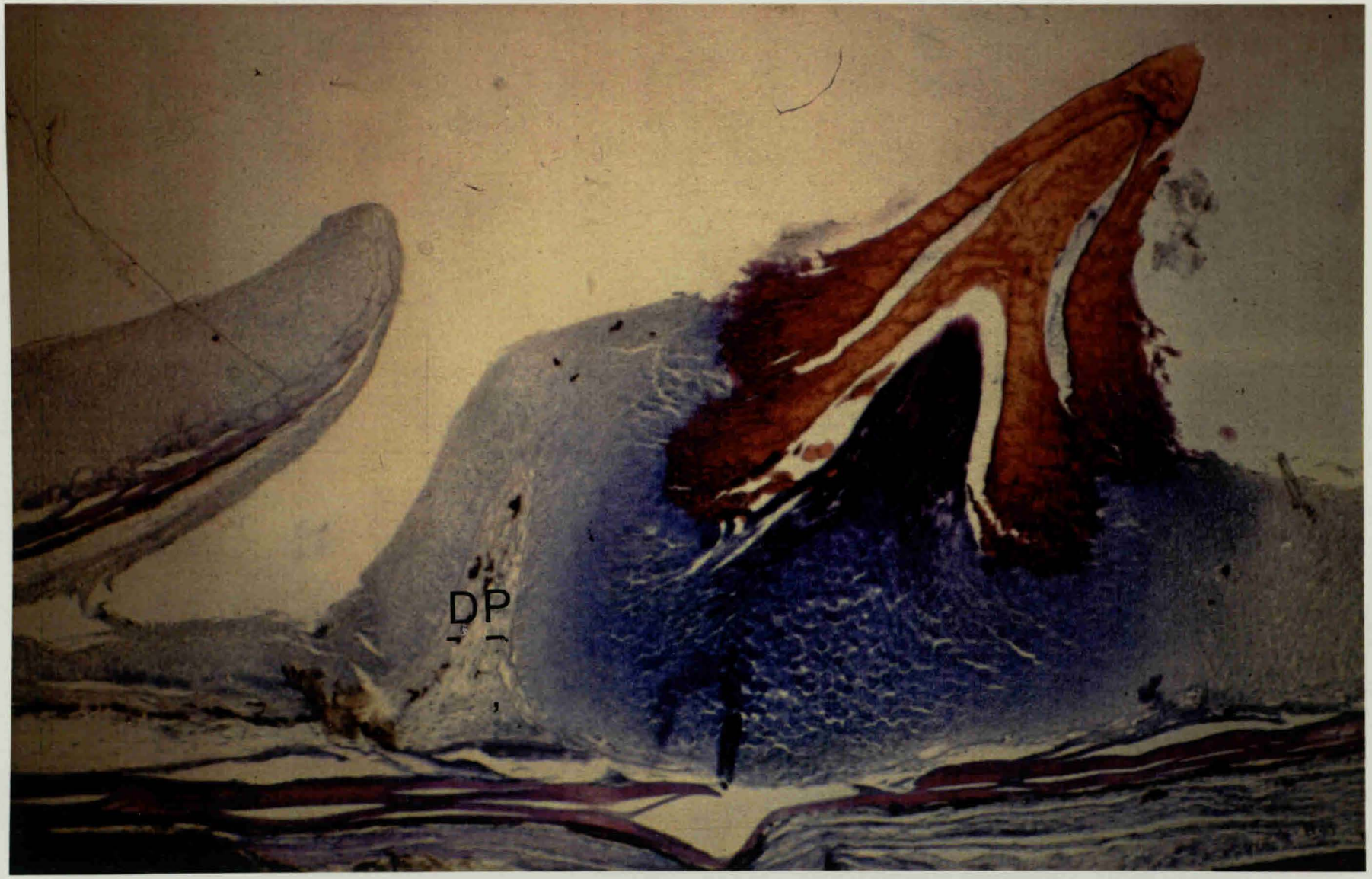
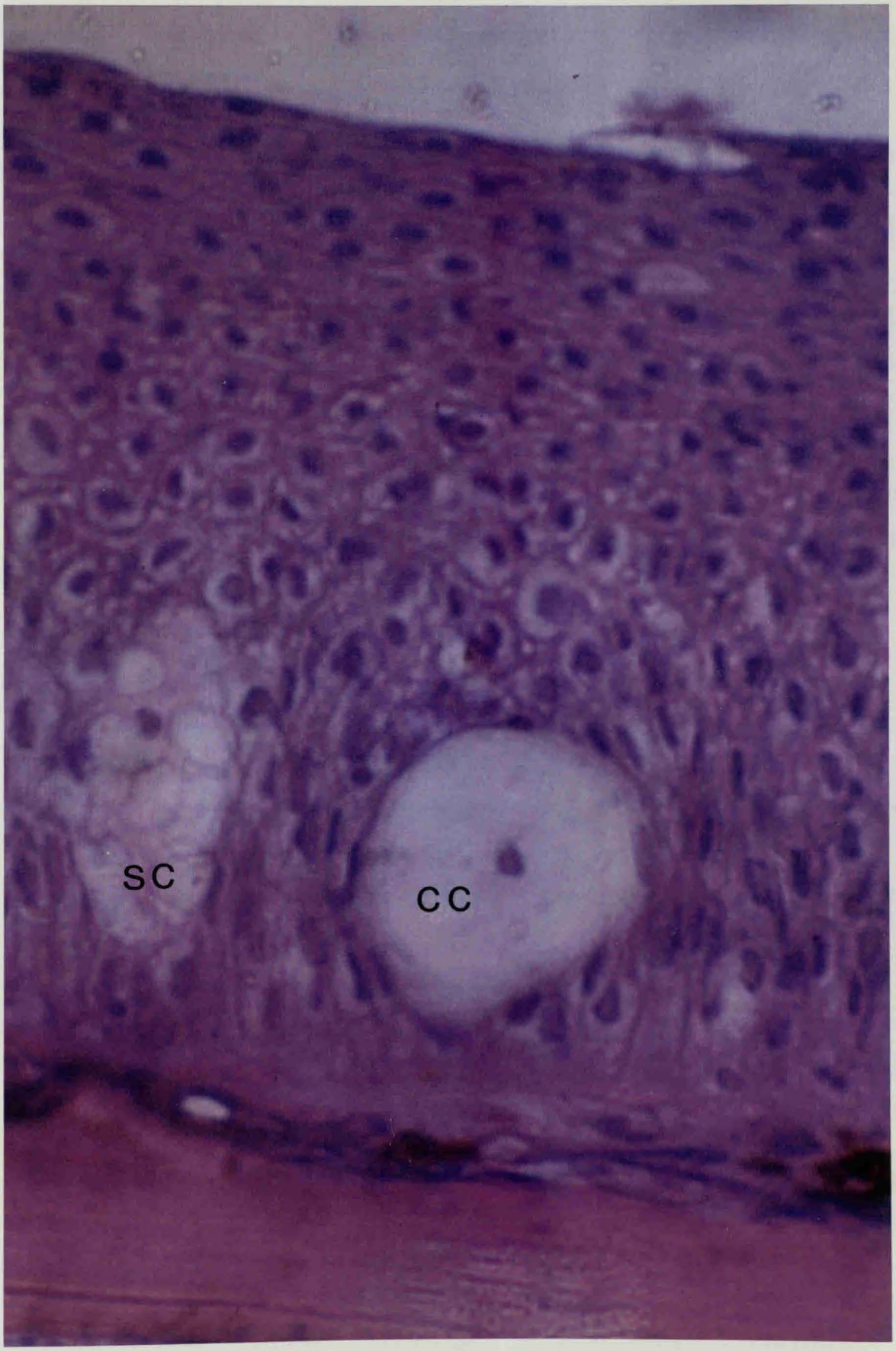

DP

Figure 21.

Light micrograph of club cells(CC), sacciform cells (SC), and cell stratification in anterior dorso-lateral trunk of *Nocomis leptocephalus* (hematoxylin and eosin, x2503).


SC
CC

Appendix

The following is a complete list of materials studied in order to find the representative samples listed in the materials and discussion sections.

The state, drainage, collection number (WSW = William S Woolcott and EGM = Eugene G. Maurakis), county, location, date, and sexual type for *Campostoma anomalum, Semotilus atromaculatus, Nocomis leptocephalus, Exoglossum maxillingua,* and *Clinostomus funduloides* are:

Fishes

Nocomis leptocephalus. Virginia: James, WSW-VA-392, Bath Co., Hot Springs Run at bridge on Co. Rt. 687 about 0.6 km N of Co. Rt. 687 and Co. Rt. 605, 21 May 1990. New, EGM-VA-256, Carrol Co., tributary of Big Island Creek, Co. Rt. 673, 0.8 km S of US 221, 2 June 1990.

Semotilus atromaculatus. Virginia: James, EGM-VA-238, Goochland Co., Genito Creek, 0.8 km W on Co. Rt. 641 off Shallow Well Road. Maryland: Potomac, EGM-MD-246, Washington Co., tributary of Little Antietam Farm Creek on property of Brookview Hospital, Leitersburg Rd. at Jct. with Durberry Rd., 2 May 1990.

Exoglossum maxillingua. Virginia, James, EGM-VA-260, Craig Co., Johns Creek, Co. Rt. 632 at Maggie, 13 June 1990.

Campostoma anomalum. Virginia: James, WSW-VA-392, Bath Co., Hot Springs Run at bridge on Co. Rt. 687 about 0.16 km N of Co. Rt. 687 and Co. Rt. 605, 21 May 1990. New, EGM-VA-252, Craig Co., Sinking Creek, St. Rt. 42 about 1.6 km S of Jct. with Co. Rt. 626, 16 May 1990.

Clinostomus funduloides. Virginia: Roanoke, EGM-VA-259, Montgomery Co., North Fork of Roanoke River, Co. Rt. 785 near Jct. Co. Rt. 1035, 4.8 km E of Blacksburg, 2 June 1990. James, EGM-VA-238, Goochland Co. Genito Creek, 0.8 km W on Co. Rt. 641 off Shallow Well Road.

Videos

Nocomis leptocephalus. North Carolina: Little Tennessee, EGM-NC-211, Macon Co., unnamed tributary at Jct. of Horse Cove Rd. and Leonard Street, 7 June 1988, tapes 1-8. Virginia: Roanoke, EGM-VA-202, Pittsylvania Co., Pumpkin Creek, St. Rt. 86 bridge at Danville City line, 11 May 1986.

Campostoma anomalum. Virginia: James, WSW-VA-383, Jackson River, 8 km W of Warm Springs on St. Rt. 39 at Meadow Lane Lodge, 28 May 1988, tapes 1, 2, and 3; WSW-VA-381, Campbell Co., Opossum Creek at bridge off Co. Rt. 669 near Jct. with Co. Rt. 680, 9 May 1988, tapes 1 and 2; New, EGM-VA-225, Craig Co., Sinking Creek, Co. Rt. 42 at bridge on Hoffman Farm about 0.8 km S of Co. Rt. 626, 26 May 1989 tapes 1 and 2.

Clinostomus funduloides. Virginia: James, EGM-VA-221, Goochland Co., east branch of Genito Creek, 0.8 km W on Co. Rt. 641 off Shallow Well Road, 17 April 1989. EGM-VA-289, Goochland Co., east branch of Genito Creek, 0.8 km W on Co. Rt. 641 off Shallow Well Road, 17 April 1992.

Vitae

William Reed McGuire was born on April 10, 1966 in Richmond, Virginia. He attended St. Christopher's School and graduated from The Asheville School in Asheville, North Carolina in 1985. After working on an oyster farm in New England, and becoming enthused with the subject of biology, he attended University of Richmond on an advanced scholars program in 1984. He studied Biology at Hampden-Sydney College where he was graduated with a Bachelors Degree in Science in December of 1988. In September of 1989, he returned to the University of Richmond to receive his Masters of Science degree in Biology in August 1992. He has taught Biology at The Episcopal High school in Alexandria, Va. and currently teaches at St. Christopher's School in Richmond.