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STUDIES ON SCALE STRUCTURE OF FLATFISHES I. THE GENUS *TRINECTES*, WITH NOTES ON RELATED FORMS

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ABSTRACT

A detailed comparison of the scales of *Trinectes maculatus* (Bloch and Schneider) and *T. inscriptus* (Gosse) has demonstrated species specific differences valid for taxonomic purposes. A complex hinged joint attaching very large cteni to the scale plate characterizes both species. The large cteni are associated with a marked increase in skin surface which, in turn, is combined with an apparent increase in the cutaneous vascular bed suggesting a secondary respiratory and excretory function. The rigid skin flaps produced by the cteni may also be associated with a hydrodynamic "spoiler" mechanism. The lateral-line canal in both species is uniquely composed of tubes lying end to end with no ancillary scale appendages.

INTRODUCTION

To date surprisingly few studies or series of studies contribute significantly to the general subject of scale morphology in relation to taxonomy of teleostean fishes. A brief statement of significant general contributions follows.

Cockerell in a long series of papers (1909a, 1909b, 1910a, 1910b, 1910c, 1910d, 1911a, 1911b, 1912, 1913a, 1913b, 1915, 1921), Cockerell and Calloway (1909a), Cockerell and Allison (1909b), and Cockerell and Moore (1910) published keys and diagnoses of the scales of several families, genera and species. Many of these

are presented with no graphic evidence and hence are inadequate for comparisons. Cockerell (1912) brought together observations and descriptions on many orders and families of both New and Old World forms. With occasional notable exceptions, such as the designation of species differences in the genus *Boleosoma*, the work offers little to support the view that lepidology is valid in species separation. He demonstrates convincingly, however, a broad range of differences in scale characters between orders, suborders, families and genera in many instances. Unfortunately, much of his work will require repeating with more complete representation and more complete documentation.

Peabody (1928; 1931) described scales of the clupeoid and anacanthine fishes respectively. She attempted to assess whether or not scale patterns were helpful in placing these fishes taxonomically, concluding that scale morphology was in general conformity with classification by other means, with certain exceptions. Her work, based on Cockerell's collection, also suffers from too sparse a representation and inadequate samples.

Chu (1935), working on Chinese cyprinids, found scales of value in characterizing subfamilies, and that they were sometimes valuable in distinguishing genera and species. This is one of two extant taxonomic studies of the scales of a large family and contributes substantially to the general problem, even though his material was derived solely from Chinese waters, McCully (1961).

Lagler (1947) developed a key to the scales of the families of Great Lakes fishes, concluding that most fundamental scale characters cross family lines and are, therefore, of little use in separating families. Due, in part, to the regional character of his work and scattered representation from several widely separated taxa, he probably missed some fundamental characters.

Kobayashi (1951) studied the value of scale characters in appraising degrees of relationship in Japanese freshwater fishes concluding that when scales were much alike, the fishes were closely related, and when different the relationship was not close, despite gross similarities. In 1952, 1953, 1954a, 1954b and 1955, he did comparative studies of the scales of this wide variety of fishes with reference to their phylogeny and probable evolution. His general conclusions have not yet been seen by us, but he clearly demonstrated the systematic value of scales.

McCully (1961) undertook a study of relationships within the Serranidae. McCully's work was based on light-microscopy studies of scales stained with Alizarin Red S. In large part, he sustained and reinforced many of the conclusions of Jordan (1923). McCully arranged subfamilies into four groups, showing possible relationships based on scale structure. Further, scale examination suggested errors in the assignment of certain genera to the Serranidae. McCully demonstrated that the existence of common morphological features can be found in the scales of divergent genera if they are related, and, conversely, that common characters are few or lacking if they are not related. He concludes that "many improvements in the systematic arrangement of the Serranidae have come to light through the [this] taxonomic study of their scales". His observations were limited to body scales and did not define ranges of variation of scale forms on a single fish or within a species.

Koo (1962) and Bilton *et al.* (1964) published methods of identification of species of Pacific salmon by scales. They did not include the masu salmon (*O. masou*) and the steelhead trout in their studies. Mosher (1969), however, included both forms in his studies on the identification of Pacific salmon and steelhead trout by scale characteristics. His illustrated guide for practical use shows clear differences in details between species of Pacific salmon (*Oncorhynchus*) and the steelhead trout. His paper is based on one by Barnaby and Delacy (1944), obviously includes much new work, and clearly establishes the usefulness of scales in definition of closely related species.

DeLamater and Courtenay (1972; In Prep.) initiated their studies on a relatively small number and a diverse selection of both closely and distantly related fishes using initially the scanning electron microscope and subsequently extending observations by application of the Alizarin Red S method for photonic microscopy. These studies have shown the mutually supportive value of using both methods simultaneously and have demonstrated the value of scale characters in separating orders, families, genera and species. Extensive evaluation of the range of variation of scale shapes and patterns on a single fish and between individuals of the same species substantiates the validity of the methods used. We have also used both body scales and lateral-line scales, demonstrating the additional wealth of characters present on the lateral-line scales (DeLamater and Courtenay, In Press).

To date, no single extensive study of the scales of flatfishes has been done, although extensive use of scales has been made in the taxonomy of these groups of fishes. A review of some of the more pertinent articles is presented below, giving a general picture of the present status of the situation relative to this important and interesting group of fishes.

Cockerell (1912) figured scales from three genera of soles: *Achirus lineatus* (Linnaeus), *Symphurus* (*Aphoristia*) *piger* (Goode and Bean), and *Solea solea* (Linnaeus). The figures, even though inadequate to characterize the genera completely, are still sufficient to demonstrate generic differences. He mentioned the genus *Cynoglossus* as having strongly ctenoid scales on the eyed side and cycloid scales on the blind side. Cockerell (1915) referred to scales of *Citharichthys spilopterus* Gunther and *Paralichthys woolmani* Jordan and Wilkins, both pleuronectids in his key. His characterizations are inadequate. Hagerman (1952) described the cycloid scales of the Dover Sole, *Microstomus pacificus* (Lockington), and indicated to a small extent the range of variation on a single specimen. Chabanaud (1928; 1929) studied achirins and the genus *Pegusa*, defining two subgenera. Scales of these fishes are ctenoid on the upper surface and cycloid below. Comparisons and diagrams of the scales of these species with those of *P. lasearis* demonstrate clear differences.

Weed (1961) notes that the scales on the eyed side of *Asseragodes ocellatus* are "weakly" ctenoid and on the blind side "weakly ctenoid or cycloid", and that the cteni do not show through the skin. Caldwell and Briggs (1957) reported on the occurrence of the naked sole, *Gymnachirus williamsoni* (Gunter), at Brunswick, Georgia. While they made no mention of embedded scales, we note reference to minute embedded and highly characteristic cycloid scales in the "naked" areas on the blind side of *Platichthys stellatus* (Pallas) (Orcutt, 1950).

House *et al.* (1970), in discussing the development of the lined sole, *Achirus lineatus* (Linnaeus), describe scale development from papilla-like structures on the sides of the larvae which absorbed and retained Alizarin stain. These papillae occurred in five rows on the larvae. The cteni or spines became longer as the larvae developed. Ctenoid scales were present on both blind and eyed sides. No ultrastructural studies on flatfish scales have yet been done.

Hubbs (1945) in his consideration of the phylogenetic position of the Citharidae notes in *Brachrypleurops axillans* Fowler (= *Citharoides menlepidotus* Hubbs) that lateral-line tubules are usually bifurcate with a T or Y shape. He further quotes Trewavas that specimens of *Paracitharus* from Natal and Zanzibar all have branched lateral-line tubules. It is of interest that Hubbs, without discussing scale characters in general for these forms, uses them to differentiate species and to show "close" relationship of citharid genera.

Andriashev (1960), in describing a new Antarctic species of *Achiropsetta* (*A. slavac*), used scales to characterize and separate this species from *A. tricholepus* Norman. The scales were described as small, extending onto the rays of all fins and the eye, and armed with a single (rarely two) vertically protruding thin

spines, giving the body a prickly character.

Ginsburg (1952), in his study of flounders of the genus *Paralichthys* and related forms, states that the cycloid or ctenoid character of the scales is of primary importance in the major division of the species comprising the genus and is of much help in the identification of species of *Paralichthys* as well as related genera. He further states that Jordan and Evermann (1898) inadequately treat this character.

Two genera which are closely related to *Paralichthys*, *Hippoglossina* and *Lioglossina*, are distinguished by the scales, ctenoid in one, cycloid in the other. This character is also of importance in forming major divisions of the species comprising the genus *Paralichthys*. Ginsburg (1952) found the presence or absence of spinules on the scales to be the most constant of all characters used in the distinction of the species with the exception of *Paralichthys aestuarius* Gilbert and Scofield, and *Hippoglossina oblonga* (Ginsburg 1926). In the latter, the number of spinuliferous scales is highly variable but such scales are always present on the peduncle on the blind side.

Concerning generic limits, the structure of the scales, i.e. cycloid or ctenoid, is of moderate use. The known species which apparently belong to *Paralichthys* either have all scales cycloid, or when ctenoid scales are present they are typically confined to the eyed side. Sometimes very few ctenoid scales are present on the blind side as a rather infrequent individual variation. If a species typically does have ctenoid scales on the blind side, it is highly probable that it does not belong to *Paralichthys*. In the known species of *Hippoglossina* ctenoid scales are either present on both sides or absent on both sides. In the described species of *Pseudorhombus*, the eyed side is ctenoid with ctenoid or cycloid on the blind side. The character is useful for subgeneric division. The species of *Paralichthys* may be divided into two apparently natural groups by the presence or absence of ctenoid scales on the eyed side. In *Pseudorhombus*, the same difference on the blind side may possibly be used for subgeneric division. In one subgenus of *Hippoglossina*, *Lioglossina*, however, this difference is only of specific importance. The structure of the scales is very constant intraspecifically with two exceptions. In *H. oblonga*, the number of ctenoid scales differs greatly with the individual. In *Paralichthys aestuarius*, the ctenoid scales lose their spinules with growth and all scales thus appear cycloid in large specimens.

The presence or absence of accessory scales was found to be a good criterion for the generic separation of the species concerned. All species of *Hippoglossina* examined lack accessory scales. All species of *Paralichthys* have accessory scales. These appear rather late in the life of the fish and this character also appears to be a late evolutionary acquisition. Because of their late appearance, the practical usefulness of this character is limited. The relative development of this character also differs much with species. It is seemingly also not of transcendent importance in generic separation. It has been neglected by authors in general and it is difficult to appraise its true value.

Gutherz (1966), in his revision of the flounder genus *Ancylosetta*, states as a generic character that scales are ctenoid on the ocular side and ctenoid, cycloid, or both, on the blind side. He defines the character of the ctenoid on four species: *A. antillarum* Gutherz, *A. microctenus* Gutherz, *A. cycloidea* Tyler, and *A. dilecta* Goode and Bean, and illustrates them, giving a good analysis of relationships of species. Tyler (1959), in describing two new species in the same genus, *A. cycloidea* and *A. kumperae*, showed *A. cycloidea* to have cycloid scales only on both sides, while *A. kumperae* was shown to have only ctenoid scales on both sides, as does *A. quadricellata* Gill to which it is closely related.

Fraser (1971), in his notes on the flatfish genus *Syacium*, refers only to a variation in "the pored lateral-line scale count". The types and distribution of the scales occurring in the four species studied are not noted.

Orcutt (1950) clearly describes the changes in the structure of the scales with increased body size in *Platichthys stellatus* (Pallas). Small fish (+ 25 mm standard length) possess minute embedded cycloid scales, which, as the fish become larger tend to develop into stellate plates with spinous tubercles. He describes the distribution of stellate scales on the blind and eyed sides as differing. Orcutt also notes the occurrence of hybrids between *P. stellatus* (Pallas) and *Parophrys vetulus* Girard, formerly described as *Inopsetta ischyra* (Schultz and Smith), just as *Pseudoplatichthys oshorensis* Hikita is a hybrid cross between *P. stellatus* and *Kareius bicoloratus* (Hubbs and Kuronuma), among others. Orcutt hybridized *Platichthys stellata* and *Perophrys veluta*, but no one to date has studied the scales in such hybrids.

Tyler (1960), in discussing differentiation of species in the genus *Poecilopsetta*, notes that in *P. inermis* (Breder) the scales are highly "deciduous" and cycloid whereas those in *P. beani* Goode are not "deciduous" and are ctenoid. *P. albamarginata* Reid likewise has ctenoid scales on the ocular side.

Starks and Thompson (1910), in reviewing flounders of the genus *Pleuronichthys*, describes the size (small or large), distribution and degree of imbrication and/or isolation of scales in the different species. They considered but did not describe or define the differences in morphology between the scales of different species.

Ginsburg (1951), in his consideration of western Atlantic tonguefishes, notes that species of *Symphurus* are unusually uniform in their structure and that not many characters are available for distinguishing species. In his generic description of this genus he states: "Body and head with ctenoid scales, those on anterior part becoming more or less embedded; caudal peduncle scaled at its base, the scales continued backward in rows between the rays for some distance, scaleless distally; dorsal and anal fins with short rows of small scales along the proximal part of the rays. Lateral-line is absent." Beyond this he gives no characterization of the scales themselves or differences between species. Bohlke (1961), in his description of two new species of *Symphurus*, provides no further elaboration on scales, their form or distribution. Robins and Randall (1965), in describing *Symphurus arawak*, a dwarf Caribbean tonguefish, note that "The scales are strongly ctenoid; the cteni are about equally developed on scales from both sides of the fish. The scales extend forward to the snout tip. They are not much embedded; in fact they are quite deciduous for a *Symphurus*." Beyond this there is no characterization of the scales present, or a statement as to whether a lateral-line is present or absent.

The inconsistent and spotty use of scale morphology in fish taxonomy is apparent from the work cited, as is the inconsistent attitude as to its importance, some authors giving it great credence, others little or none.

MATERIALS AND METHODS

The following two species of *Trinectes*, *Trinectes inscriptus* (Gosse) and *T. maculatus* (Bloch and Schneider), were made available for this study by C. R. Robins and W. R. Courtenay, Jr. respectively. Max Hancock kindly supplied specimens of *Lepidopsetta*. Robins also kindly supplied the specimens of several species each of *Ancylopsetta* and *Citharichthys*; Courtenay, the specimens of *Platichthys stellata* (Pallas) referred to.

Observations were made by means of both the ISI Miniscan scanning electron microscope and with either a Reichert photonic microscope with or without an "epilum" (surface viewing) attachment and a 35 mm camera or with a B&L reverse stand research microscope set on a B&L - L camera using 5 x 7 photographic film and with the camera set at 20 inches above the microscope to achieve 2X multiplication of the microscopic magnification. A range of 3.5X to

40X objectives were used and paired with a range of 5X to 28X oculars to obtain desired magnifications. Light source for the Reichert system was a mercury vapor arc lamp or an alternative tungsten source. For the B&L system, a 100W Zirconium arc lamp was used set at a distance to achieve Koehler illumination. In each light path a ground glass filter was inserted to achieve certain effects or removed to obtain others. In general, the ground glass filter eliminated diffractions and increased certain details, particularly of bony structures. It likewise obliterated phase differences and therefore wiped out certain delicate details, particularly of soft tissues, which were sometimes desired.

Kodak Ectopan and DuPont Cronar graphic 5 x 7 cut film were used in the B&L system; Kodak Plus X 35 mm film in the Reichert system. Polaroid PN55 film was used exclusively in the scanning instrument. Here the prints were processed and the negatives cleared and used in producing photographic prints for study, comparison and publication.

Each system, scanning and photonic microscopy, required a separate method for preparation of material for observation. Scales for observation in the scanning electron microscope were first cleaned by physically removing as much adhering tissue debris as possible without touching or damaging the scale surface. They were then soaked in a solution 10% H₂O₂ for several hours to bleach chromatophores and to soften adhering tissues. They were then soaked for 12 to 24 hours in saturated borax solution with trypsin (1 teaspoonful per ½ pint) to clear them, and subsequently sonicated gently in a Desontegrator, Model #T4C1, sonicator to finalize the cleaning procedure. It was also found that a few minutes exposure of specimens to 10% KOH effectively loosened adhering tissues and speeded the cleaning and clearing procedure. They were then dried rapidly through a series of alcohol to 100%, blotted free of solvent and pressed flat on the metal specimen pegs to which a small piece of double stick tape had been applied. Electrical contact with the peg was insured by touching a small drop of aluminum paint between the specimen and peg at one point. If a piece of skin carrying scales was the specimen, it was handled and mounted in the same way.

Specimens for white light microscopy were cleaned in the same manner and cleared by exposure to the borate-trypsin solution. They were then stained in 5cc of 0.05% KOH to which 5cc of Alizarin Red S solution was added. After staining for 8-10 minutes or longer as needed, the specimens were passed through 25% glycerin, 50% glycerin and 75% glycerin into 100% glycerin and mounted in 100% glycerin on glass slides. The specimens were held flat with 1-2 oz. lead weights and sealed with nail polish. Both individual scales and skin carrying scales were handled in the same manner.

OBSERVATIONS

Descriptions of Scales and Cteni

Trinectes maculatus.—Viewed with the photonic microscope, the scales in *T. maculatus* are strongly ctenoid on both the eyed and blind sides. The size of the scales on both sides varies widely from very small, bearing 1, 2, 3 or more cteni (Figure 1A), increasing in size directly with the number of cteni. The largest scales may bear 15 or more cteni (Figure 1B). The small scales appear to be secondary or accessory to the larger scales frequently overlying the scale plates of larger forms (Figure 1A). The scale plate shapes vary from almost round to ovate to almost rectangular or triangular with the posterior border bearing the cteni indented, to straight, to outwardly curved posteriorly (Figure 1).

In general, the pattern of the circuli appears to be consistent from one scale form to another; the radii are however much more fully formed, that is, they are deep radial clefts in the calcified layer in the smaller scales, proceeding from the

focus anteriorly, dorsoanteriorly and ventroanteriorly (Figures 1A, C). In the larger scale forms, the radii may occur only as gentle undulations in the scale plate rather than as clefts in the calcified tissues, or they may be lacking altogether (Figures 1B, D).

Although the scales vary in size, the basic body scales producing the primary pattern of imbrication are what might be called middle size having 5, 6, 7 or 8 large cteni. These may be overlaid or have interspersed between them much smaller scales from 1 to 4 proportionately large cteni (Figure 1A).

Scales are continued along the fin rays (Figure 1C). Quite consistently there is a row of larger scales which decrease in size progressively toward the tips of the fin rays, which have a consistent row of much smaller scales laying anteriorly over their scale plates.

At the bases of the dorsal and ventral fin rays, there are arrays of frequently very large scales often carrying as many as 15 or more gigantic cteni (Figures 1B, 2A). On the caudal peduncle and fin, the scales become progressively smaller posteriorly. Just posterior to the opening of the operculum, there is a row of very large scales with trianguloid scale plates and relatively tremendous cteni (Figure 1D).

The scale is formed of a scale plate made up medially (under surface) of criss-cross laminations of connective tissue fibers overlaid by calcified tissues in which the circuli and radii are formed. The focus of the scale in *T. maculatus* lies well posteriorly just anterior to the posterior border of the plate which is thickened as an articulating surface against which the cteni articulate (Figures 2A, B, D).

The cteni are complex and composed of two discrete components: The cteni themselves which are long tapering spike-like bones with well formed bifurcate articulating joints at their anterior ends where they articulate with, well formed intercalary bones (Figures 2A, B, C, D). These are rounded posteriorly where they articulate with the larger cteni and bifurcate anteriorly into two articulation processes with a medial ridge between them. They articulate either with additional ranks of similar intercalary bones or with the articulation ridge of the scale plate itself. These complex joints are reminiscent of the wrist joint in humans with its multiplicity of small interarticulating bones. There is no question but what these joints are movable since they can be moved with the tip of a needle. They appear to be held together and to the scale plate by connective tissue fibers. Sectioned material indicates that the cteni are held in a sheath of connective tissue covered by epithelial tissues. To date we have obtained no evidence of an inherent musculature which could be used to voluntarily move, i.e. raise and lower, the cteni. Muscle fiber attachments from the myomeres which pass through loops of connective tissue on the medial surface of the dense connective tissue layer of the dermis have been observed which when pulled wrinkle the skin and thus are capable of raising the scales and so elevating the cteni.

Scanning electron microscopy of these scales confirms and extends the observations by the Alizarin Red S method. The staining procedure has the advantage that due to the specific calcium staining, it is possible to visualize structures at least at low resolution that are covered or embedded in the dense connective tissue layers of the dermis, as the scales in this species are. With the scanning electron microscope, the connective tissue matrix, which is very difficult to remove in this species, frequently obscures observations of desired details.

Where this connective tissue matrix has been removed, the following supporting observations are clear (Figures 2B, D): (1) The crests of the circuli are smooth and carry no tubercles or denticles as in other groups of fishes such as the tilapias (DeLamater *et al.*, 1972); (2) The structure and organization of the cteni, intercalary bones and the articulation ridge of the scale plate are further clarified. The articulation ridge along the posterior border of the scale plate is

much thickened frequently incorporating specialized articulations for the intercalary bones and appearing as though they had been formed by the incorporation by fusion of intercalary bones to the scale plate, implying, perhaps, that growth may involve elaboration of new intercalary bones between the cteni and the articulation ridge of the scale plate. The articulation surfaces are relatively smooth, but the medial ridge lying behind and between these is surprisingly porous (Figure 2D). The basal or anterior parts of the cteni also carry numerous pits and indentations on their lateral surfaces distal or posterior to the articulations (Figures 2B, C, D).

Dislocation results in disarticulation of the cteni, intercalary bones and articulation ridge of the scale plate showing their characteristic shapes and relative sizes (Figures 2A, C). Fracture of a ctenus is strongly reminiscent of fracture of true bone (Figure 2C) but in miniature, reminding one of the mesodermal origin of these structures.

All four specimens of *T. maculatus* examined were consistent in the features described.

The size and complexity of these scale structures are characteristic of *T. maculatus* and can be used in the definition of species as will be seen in the comparison with *T. inscriptus* which follows.

Trinectes inscriptus.—As in *T. maculatus*, this species has strongly ctenoid scales on both the eyed and blind sides. The scales of *T. inscriptus* viewed by photonic microscopy after clearing and staining with Alizarin Red S are overall very similar to, but much simpler than, those of *T. maculatus* (Figure 3). The body scales are very regularly arranged in an imbricate pattern (Figure 3A). At least on the specimens examined, unlike *T. maculatus*, few accessory scales were present either on the body or fin rays, but when present, occurred toward the bases of the fin rays. The numbers of cteni are also much reduced, the range being from one in the smaller occasional secondary scales to 6 or 7 on the largest scales, again the largest and most complicated being near or toward the bases of the fin rays (Figures 3C, D). Along the fin rays, the scales become progressively and regularly smaller and less complicated from the bases toward the tips (Figure 3E).

The pattern of both the circuli and the radii are very similar to those seen in *T. maculatus*. When present, the radii occur as clefts or disruptions in the pattern of the circuli.

The greater regularity of the scales in this species is striking. The row of large triangular multictenate scales occurring just posterior to the operculum does not occur as in *T. maculatus* (Figure 1D).

The structure of the scales in *T. inscriptus*, though simpler, has the same basic organization as in *T. maculatus*. The differences are primarily concerned with the reduced number of the cteni present and the reduced number and complexity of intercalary bones when present.

The large cteni may articulate directly with the articulation ridge of the scale plate or they may articulate with intercalary bones which in turn, articulate with the articulation ridge of the scale plates. Only rarely is there more than one rank of intercalary bones present in contrast to *T. maculatus* (Figures 3A-F).

The structure of the cteni and their articulating processes are very similar to those in *T. maculatus*. The shafts of the cteni are not in general so robust, however. The structure and shape of the intercalary bones can be very similar to those of *T. maculatus* with the characteristic dual articulation processes, but are more flattened on the rounded posterior surface which articulates with the cteni. They are as a consequence thinner and flatter. From this more complex structure they may occur as reduced flattened discs lying against the articulating ridge with minimal or absent articulating processes (Figure 3D), or they may be absent entirely.

These characteristics were present in all three specimens of this species examined and appear to constitute a valid basis for the separation of these two species.

Scanning electron microscopy studies of scales of *T. inscriptus* again confirm and extend the observations by photonic microscopy. The forest of cteni are clearly shown in Figure 2F, as is the remaining persistent connective tissue between the cteni and the dermal connective tissue layer within which the scales are embedded.

The articulations between cteni and the articulation ridge of the scale plate, both with and without a flattened disc-like intercalary bone are clearly shown in Figures 3A and F.

Lateral-line Canal.—The lateral-line canal is very similar in structure in both species and appears to be the same on both the eyed and blind sides in both, unlike other species examined and to be reported elsewhere. For purposes of brevity, this structure will be described simultaneously for both species even though future studies may reveal differences.

The lateral-line canal is composed of a continuous row (series) of individual tubes strung end to end, the posterior end of the next anterior tube lying slightly above the opening of the next posterior tube and tapered somewhat to fit into it. At these successive junctures, there is an opening toward the lateral or outer side formed by the shape of the roof of each tubule, i.e. by the way each is cut back (Figures 2E, 3A). The canal, built in this disjunctive manner of a string of individual tubes, is continuous in a rather straight line from a midpoint just behind the head to the tip of the caudal fin. This jointed canal obviously allows lateral (dorsoventral in a flatfish) movement. The individual tubes, unlike those in other flatfishes so far studied, do not have ancillary structures such as scale plates indicating their origin from scales [see Figure 4A, *Lepidopsetta bilineata* (Ayres)]. The whole lateral-line canal is deeply embedded in the thick and dense connective tissue matrix of the dermis (Figures 2E, 3A, B) and is not directly associated with any scales. The scales, lying over the canal lie above it, are also embedded in the connective tissue matrix but are separate and more peripheral (i.e. closer to the surface).

Many of the individual tubules of the canal possess dorsal and ventral (lateral) pores as seen in Figure 2E. Their function is not clear but they may be present for the admission of blood vessels, etc., as suggested by sectioned material.

It is presently presumed, on the basis of current studies on other flatfishes, that connections exist between the interior of the canal and the outside in the form of soft tissue tubes as in Figures 4A and B, *Lepidopsetta bilineata*, or by direct soft tissue continuations of the posterior end of each tubule to the surface as in *Citharichthys cornutus* (Gunther) (Figure 4F). Further studies are in progress to define the ranges of this type of structure that may occur in this remarkable group of fishes.

Membranous covering of the cteni.—By viewing the skin surface directly with reflected light at low to moderate magnifications, it is clear that the cteni and their covering of dermis project from the skin surface forming flat palmate projections or flaps held rigid by the cteni. Figure 2F shows a scanning electron microscopy view of the cteni projecting from the skin surface. Figure 4D shows the epidermal covering of comparable much enlarged cteni in *Lepidopsetta* by photonic microscopy. It is obvious that the surface of this skin is greatly increased by the folding produced by covering both outer and medial sides of the projecting cteni. It was therefore of considerable interest that in both preparations for scanning and photonic microscopy small arterioles, veinules and capillaries could be demonstrated not only in the epidermis covering the scale plates but also in the membranes between the cteni, as shown in Figure 4C of *Lepidopsetta bilineata* and Figure 4E of *Trinectes inscriptus* respectively.

DISCUSSION

It is clear from the differences shown between the scale structures of *T. maculatus* and *T. inscriptus* that these two species of a single genus can be easily recognized. It is equally clear that details of scale morphology can be a very real addition to taxonomic methodology, permitting rapid and easy recognition of species even in the absence of the whole fish.

It is apparent from the brief review of literature cited that scales have long been used in the characterization of the flatfishes and that frequently they have been used in specific differentiation. We here would like to emphasize the value of the scale characters in separating taxa and further emphasize the value of the mutually supportive methods used in these studies.

As to the possible function or functions of the greatly enlarged articulating cteni in the species studied, we were concerned with four basic possibilities, the first of which might have a dual purpose.

The cteni of both species are jointed (articulate) and are movable, but at present there is no evidence for an intrinsic musculature permitting independent movement of the cteni or clusters of cteni on a single scale. The muscular mechanism is available, however, for the wrinkling of the skin and therefore for the tilting or changing the angle of the scales and the cteni.

It was thought that independent movement might be related to potential displacement of parasites on a bottom-dwelling organism or to dispersal of sand or sediments in efforts to camouflage. There is, however, no evidence as yet to support either notion.

The wrinkling of the skin could however make use of the rigidly held skin flaps to effect a change in skin surface to produce a spoiler effect on smooth hydrodynamic flow thus producing a braking action. This idea is amenable to study and would be warranted on the basis of the physical characteristics of the skin surface described herein.

The fact that the palmate projections of the covering membranes produced by the projecting clusters of cteni from each scale are associated with a relatively tremendous increase in skin surface and simultaneously with an increase in the capillary bed suggests that these structures may be associated, in certain forms at least, with an accessory cutaneous respiratory and excretory mechanism to supplement gill respiration in bottom-dwelling fishes whose gill function might be restricted or interfered with by bottom sands and sediments.

This idea is also amenable to experimental evaluation and one which, if proven out, would give further insight into the habit, behavior and physiology of this fascinating group of fishes.

The elaborate joints in the scales of these fishes also appear to be related to the flexibility of the body allowing extensive bending of the scale without fracture. The degree of overlap of the cteni over the next posterior scales might obstruct free bending were the joint not present.

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Figure 1. *Trinectes maculatus* body scales - photonic microscopy - with ground glass.

- A Body scale pattern and imbrication showing variable scale sizes and variable numbers of cteni.
- B Large triangular ctenate body scales with complex joints with intercalary bones.
- C Scales over ventral fin ray.
- D Large scales in row posterior to operculum.
- E Small round secondary body scale.



Figure 2.

A & C *Trinectes maculatus* body scales, photonic microscopy with ground glass.

B & D *Trinectes maculatus* body scales, scanning electron microscopy.

E Lateral-line canal *T. maculatus*, photonic microscopy with ground glass.

F *T. inscriptus* body scales, scanning electron microscopy.

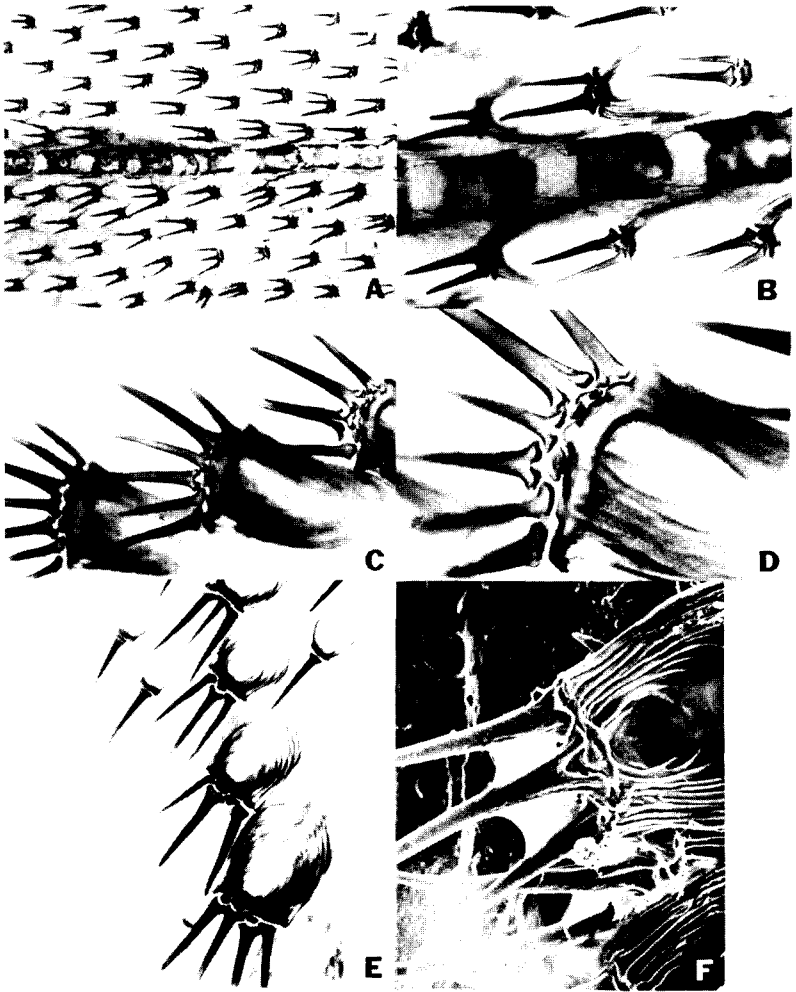


Figure 3. *Trinectes incriptus*. A-E, photonic microscopy, with ground glass; F, scanning electron microscopy.

- A Body scale pattern and imbrication with lateral-line canal.
- B Enlargement for detail of scales and canal.
- C Large body scales at base of fin ray for cteni and intercalary bones.
- D Enlargement for details of ctenal bases and intercalary bones.
- E Scales over fin ray.
- F Scanning electron microscopy for detail of cteni intercalary bones and articulation ridge of scale plates and circuli on scale plates.

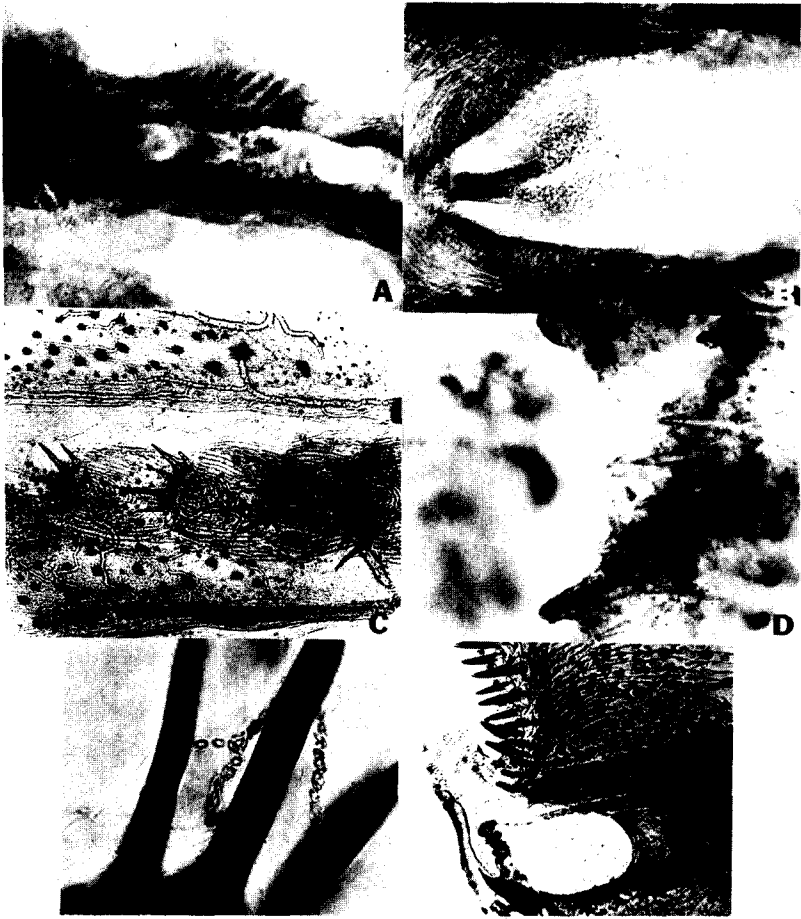


Figure 4.

- A *Lepidopsetta bilineata*. lateral-line scales showing soft tissue tubules leading from canal to exterior. Photonic microscopy without ground glass.
- B *Lepidopsetta bilineata*. enlargement of soft tissue tubule of lateral-line system. Photonic microscopy without ground glass.
- C *Ancylosetta quadrocellata*. vascular bed over and adjacent to scales. Photonic microscopy without ground glass.
- D *Lepidopsetta bilineata*. epidermal covering of cteni. Photonic microscopy with ground glass.
- E *Trinectes inscriptus*. capillaries in membranes between cteni. Scanning electron microscopy.
- F *Citharichthys caruntus*. soft tissue extension of posterior end of lateral-line canal. Photonic microscopy without ground glass.

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**SEPARATION OF THE SUBSPECIES OF
LARGEMOUTH BASS *MICROPTERUS SALMOIDES*
SALMOIDES, AND *M. S. FLORIDANUS* AND
INTERGRADES BY USE OF MERISTIC CHARACTERS**

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ABSTRACT

Meristic variation was examined among samples of northern largemouth bass, *Micropterus salmoides salmoides*, from Arkansas and compared with variation found in samples of the Florida largemouth, *Micropterus salmoides floridanus*. Meristic characters employed in this investigation included five scale counts and number of pyloric caeca. Statistical analyses of the data involved analysis of variance, discriminant function analysis, and Hotelling's T² test statistic. The primary objective of this study was to establish the best criteria for the practical separation of the two subspecies and, if possible, their intergrades. Number of pyloric caeca, which had not been previously compared for these two subspecies, proved to be the best single character for their separation. Utilizing two scale characters and number of pyloric caeca, discriminant function analyses permit classification of unknown specimens to one or the other subspecies with a high degree of certainty. A graph, containing two overlapping ellipses, constructed using Hotelling's T² test statistic, enables a rapid classification of *M. s. salmoides*, *M. s. floridanus*, and possibly intergrades by plotting original values for number of lateral-line scales and number of pyloric caeca on the axes.