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The comparative osteology of the trunk skeletons of three species of Paralichthys, family Bothidae, from North Carolina

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The Comparative Osteology of the Trunk Skeletons of Three Species of *Paralichthys*, Family Bothidae, from North Carolina

A Thesis

Presented to the Faculty of the Graduate School

of the University of Richmond

in Partial Fulfillment of the Requirements for the

Degree of Master of Science

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Introduction

Paralichthys albigutta Jordan and Gilbert, the gulf flounder, which occurs over sand bottoms, and *Paralichthys lethostigma* Jordan and Gilbert, the southern flounder, which occurs over mud bottoms, are southern species with ranges extending into the Gulf of Mexico (Ginsburg, 1952). *Paralichthys dentatus* (Linnaeus), the summer flounder, is the northern species and is tolerant of several types of bottoms but is most frequently found over sand or hard bottoms (Ginsburg, 1952). The ranges of the three species overlap in an area along the eastern coast of the United States from central North Carolina to near Jacksonville, Florida. The flounders are closely related and unless the pigment pattern is well developed identification ~~if~~ is difficult (Ginsburg, 1952). The difficulties are magnified where the ranges of the species overlap.

Pigment pattern does not develope until a size of about 40 mm is reached; smaller specimens frequently are problems in identification. Easier species identification of young flounders would be of economic advantage as two of the species (*P. dentatus* and *P. lethostigma*) are of considerable importance as food fishes. Life history studies of these flounders have been particularly difficult because of the confusion in identification.

The existence of three separate species was not fully acknowledged until Ginsburg (1952) separated them on the bases of gill raker, anal and dorsal ray counts or by a correlation of these. Hildebrand and Cable (1931), using external characters, were unable to separate the fishes present at Beaufort, North Carolina into more than two species; and even though Norman (1934) did tentatively treat them as three species, he felt that *P. albigutta* was perhaps identical with *P.*

lethostigma.

The present study was therefore undertaken to determine the changes that take place in the trunk skeletons of these species between the sizes of 10 mm and 60 mm, and to see if osteological characters could be used in species separation. As the study includes specimens from only a small portion of their range, the work also serves as a preliminary study to a more comprehensive one on races of each species throughout their ranges.

Materials and Methods

Specimens were cleared and stained following the published methods of Taylor (1967) and used by Beirne (1966), with the exception that all preparations were made at room temperature (25-28 C). The first step, that of bleaching, was found to be most critical as over-bleaching led to destruction of connective tissue and subsequent disintegration of the specimen. Proper bleaching required one to four hours and was terminated when most of the pigment was removed. Clearing and staining took from one to three days for smaller specimens (10-25 mm st. lg.) whereas larger ones (30-50 mm st. lg.) required 10 to 20 days. Some of the largest specimens took up to 30 days due to the presence of preservatives that probably acted as enzyme inhibitors. The inhibitors were removed by placing the fish in 1% KOH for two to four days.

One hundred-nineteen specimens were used of which 86 were cleared and stained. Osteological observations were restricted to specimens in the range of 10-60 mm st. lg. In general, observations were made on intact organisms; however, several individuals of each species were disarticulated for study. Examinations and drawings were made with the assistance of a Wild M-5 Stereomicroscope equipped with a drawing tube.

Measurements were made with dial calipers to the nearest 0.1 mm. Counts and dimensions were taken from the eyed (left) side, except for dorsal pterygiophore and lateral-line scale counts, the former being made on the right side and the latter on both sides of the specimen. Lateral-line scale counts were made along the straight portion of the lateral-line beginning with the terminus of the hypural and ending with the last scale that approximated the horizontal before the line turned upward. Where fused pterygiophores appeared, the individual distal ends were discernible and each was counted separately. The urostyle was included in all vertebral counts. Those vertebrae anterior to the vertebra supporting the abdominal rod were considered trunk vertebrae and were counted apart from the remaining (caudal) vertebrae. The total numbers of vertebrae were used in the statistical analysis. All data were treated statistically by comparing standard deviations and by the percentage differentiation method proposed by Ginsburg (1938). Most of the means included in the text were rounded to the nearest whole number.

Specimens used in the study are listed below. Data are given in the following order: species; in parentheses, range of standard length in millimeters; museum catalogue number (UNC indicates the University of North Carolina); in parentheses, number of specimens used from that collection; locality of collection; and date of collection.

Paralichthys dentatus. (17-127). UNC 3520 (21) North River, N. C. 17 July 1960; UNC 960 (23) Oregon Inlet, N. C. 9 March 1957.

Paralichthys lethostigma. (20-113). UNC 3354 (19) New River, N. C. 25 May 1959; UNC 2075 (15) White Oak River, N. C. 14 May 1958; UNC 2174 (13) Neuse River, N. C. 28 April 1958; UNC 3523 (4) North River, N. C. 17 July 1960.

Paralichthys albigutta. (16-130). UNC 3355 (15) North River, N. C.

17 July 1960; UNC 632 (4) Bogue Sound, N. C. 22 March 1956; UNC 3524 (3) Bogue Sound, N. C. No record; UNC 3905 (2) Bogue Sound, N. C. 15 July 1964.

Results and Discussion

External Meristic Characters. Of the characters counted, only scales in the straight part of the lateral-line, and dorsal and anal fin ray counts were found to be significant in separating the species. Both right and left lateral-line counts were useful in separation. The blind (right) side averaged about 10 more scales per species (Table 1). On the eyed side, P. dentatus had a mean of about 67 scales, P. lethostigma approximately 62, and P. albigutta 50. Separation of P. dentatus and P. lethostigma was made at the line between 64 and 65 with 82% divergence. Only slight overlap existed between P. albigutta and P. lethostigma, as an average divergence of 97% was obtained with a line drawn between 55 and 56 scales (Table 2A). The method of counting scales differed from that of Ginsburg (1952) who counted oblique rows of scales above the lateral-line, because numerous accessory scales on the lateral-line made accurate counting difficult; in larvae there were few such scales. The small differences in the results of Ginsburg's counts and those obtained by me are probably due to Ginsburg's more extensive sampling.

Anal fin ray counts gave 100% separation of P. albigutta ($\bar{x}$ 60) from P. lethostigma and P. dentatus, which had identical means of 68 rays each (Table 2C). Similar results were obtained when the dorsal fin ray counts were used. Counts of the dorsal fin rays were made from the blind side as the dorsal fin at the level of the head was displaced to the right, thus the fin passed to the right side of the eye. Its anterior-most ray was located immediately behind the posterior nostril of the blind side. The means of P. dentatus and P. lethostigma were about 88 and 87 respectively; P. albigutta had a mean of approximately 78 rays. There was a small overlap between P. albigutta and P. lethostigma and a divergence of 97% resulted from drawing a line between 82 and 83 dorsal fin rays (Table 2B).

Osteology of Trunk Skeleton. The number of vertebrae (including the urostyle) was found to be the most useful osteological character in separating P. dentatus from the other two species. Paralichthys dentatus averaged 41.2 (11 + 30) vertebrae, S. D. 0.40; the others approximated 37.0 (10 + 27), S. D. 0.35 (Table 3A). With one exception, where one P. albigutta had nine trunk vertebrae, all vertebral number variations occurred in the caudal region. In general, the vertebrae resembled those found in most teleosts, however, the first caudal vertebra was considerably modified. Its haemal arch and spine were much heavier than others in the column and served as a support structure for the abdominal rod (Fig. 1C). The haemal spine was grooved anteriorly and the abdominal rod fitted into it in a tongue-in-groove fashion. This gave the two structures a degree of unity and supplied lateral support to the rod and consequently to the body cavity. The abdominal rod was longitudinally grooved posteriorly and received the proximal ends of the six or seven most anterior ventral pterygiophores. In addition, the first ventral pterygiophore was fused to the anterior end of the rod (Fig. 1A, B, C). In the youngest specimens the vertebral centra were almost cylindrical but gradually attained the typical hour-glass shape of the adult by the time a length of 40-50 mm was reached (Fig. 1A, B, C). Concurrently the parapophyses and the neural arches of the first four vertebrae thickened and increased in bulk. The angle between the abdominal rod and the haemal spine lessened, and the distal tip of the rod curved ventrally to a position where a ventral fin ray was supported by it. Parapophyses were found from the earliest stages on all trunk vertebrae posterior to the fourth vertebra. The first pair of parapophyses were small whereas those following progressively increased in size.

Characteristically the haemal arch was completely formed at the seventh trunk vertebra in all three species, however, one specimen in 20 counted did not have a complete arch until the eighth.

Pterygiophores averaged about 20 less per species on the anal surface than on the dorsal surface, and were smallest in number in P. albigutta ($\bar{x}$ 58). Paralichthys dentatus and P. lethostigma had means approximating 66. A 100% separation of P. albigutta from the other two occurred with a line drawn between 61 and 62 anal pterygiophores (Table 3C), and a line between 81 and 82 dorsal pterygiophores gave a 97% separation (Table 3B). Each pterygiophore supported a single fin ray. In addition to the previously noted fusion in the ventral area, the first two dorsal pterygiophores were fused to each other. In very young (10-15 mm) flounders the pterygiophores were simple rods (Fig. 1A) and later the distal ends distended into a fan-like plane (Fig. 1B). When the larvae reached a length of about 35 mm, thin flanges oriented anterio-posteriad appeared immediately proximal to the flattened tips of the pterygiophores (Fig. 1C). These planar structures probably lend strength to the parent rods and lateral rigidity to the bodies of the fish.

There were eight pairs of pleural ribs in P. albigutta and P. lethostigma and nine in P. dentatus. The first two pairs were relatively small; the other six were larger but decreased in size toward the posterior end of the fish. The first two pairs (on trunk vertebrae three and four) were attached to their respective vertebral centra; the remaining ones articulated with parapophyses.

Paralichthys dentatus usually had more epipleural ribs ($\bar{x}$ 10.5) than did the other two species ($\bar{x}$ about 9.6). In all three flounders the last epipleural rib was sometimes unpaired (in 19 out of 61 specimens). Although the extra rib was found on either side, it occurred more frequently on the blind side (3:1). The first pair of epipleurals was on the second trunk vertebra and was joined to the neural arch just above the centrum. The epipleurals of the third and fourth vertebrae were affixed to the centra of their respective vertebrae. The following pairs articulated with parapophyses of successive vertebrae at points proximal to the pleural ribs. The extra pleural and epipleural ribs in P. dentatus can be correlated with the additional vertebra in the trunk region.

The caudal area of the three species was essentially identical, and in all cases there were 18 caudal fin rays (10 epaxial and 8 hypaxial). The dorsal-most ray was small and could easily be overlooked if cleaned or untreated specimens were examined. The ventral-most ray was shorter and heavier than the others and bore a small spur on its ventral surface near the point of attachment (Figs. 2 and 3). The neural and haemal spines of the penultimate vertebra were modified into the first epural and first hypural elements respectively; each supported a single fin ray. The first epaxial ray was sometimes supported by the second epural bone instead of the first (Fig. 3B), but the hypural from the penultimate always supported a ray. Below the centrum of the urostyle was an autogenous hypural which supported two hypaxial rays (Fig. 3B). Similarly there were two autogenous epurals above the urostylar centrum, which together supported three epaxial rays (Fig. 3B). The urostyle was made up of the anterior cup of a typical hour-glass centrum and was fused caudally with a large epural and a large hypural bone mass (Fig. 3B). The dorsal mass was divided into six epurals, which were fused anteriorly, and had six rays attached; the ventral mass was split into five hypural elements and supported an equal number of rays (Fig. 3B). In very small specimens (10-11 mm) the large epurals, as well as the hypurals, were solid units and not fused to the urostyle. The urostyle at this stage had an upturned vestige, which continued caudad, giving the tail a slight heterocercal appearance (Fig. 2A); the autogenous bony elements were relatively small. ~~Then~~ When the fish reached a size of 20-30 mm the urostylar process had shortened, the autogenous structures had increased in size and stress lines had appeared on the large epural and hypural bodies (Fig. 2B). Fusion of the large bones to each other and to the urostyle had taken place by the time a length of 40 mm was attained. Further changes evident were: the appearance of fissures in the newly anastomosed structures; a continued increase in the relative size of the autogenous elements; and an additional reduction in the prominence of the urostylar process (Fig. 3A). All trends in metamorphoses continued until a condition approaching that of the adult form was reached in specimens of about 50 mm length (Fig. 3B).

The pelvic girdle consisted of paired basipterygia, each of which bore 10-13 rays. *Paralichthys dentatus* and *P. lethostigma* ~~usually~~ had ~~a~~ modes of 12 rays per side, although occasional specimens had 11 or 13. In *P. albigutta* the mode per side was 11; some had 10, 12 or 13. In all three species, specimens were found with unequal numbers of rays on the right and left sides. In 40 mm fishes the posterior border of the basipterygium was marked by a heavy ridge, which in turn bore a short lateral spur (Fig. 1C). The girdle formed by a pair of these bones was inserted anteriorly into the cradle formed by the left and right cleithral bones of the pectoral girdles (Fig. 1C); the pelvics contacted each other only at their anterior tips. In younger specimens the basipterygium appeared as a rod bearing a spur (Fig. 1A), later the rod thinned and the spur became more pronounced (Fig. 1B). Finally, the typical flange along the anterior border ossified and was easily visible (Fig. 1C).

The pectoral girdle consisted of paired elements and was much like those of other flatfishes illustrated by Norman (1934). In 10 mm flounders the cleithrum was a thin elongated bone with an angle of about 140 degrees nearest its dorsal tip; the dorsal part of the bone was less than one-half (2:5) the length of the ventral part. The superior and inferior postcleithral bones were strongly ossified and had already assumed a configuration much like that found in adult fishes. Neither the coracoid nor the scapula were visible at this age (Fig. 1A). In a 30 mm specimen the angle in the cleithrum was 125 degrees and, due to a disparity in area growth rates, the dorsal part was then fully one-half (1:2) as long as the ventral portion. The scapula and coracoid were partly ossified; the scapula appeared as a flattened bony plate with a slender caudad process; the coracoid resembled a rod that was compressed laterally at its dorsal end. The two bones appeared to have developed as separate centers of ossification in a single cartilage matrix (Fig. 1B). Metamorphosis of the cleithrum was nearly complete in fishes 40-50 mm in length. Further unequal growth of the cleithrum occurred until the dorsal and ventral parts were approximately equal in length (4:5) and the angle approached 120 degrees (Fig. 1C). The scapula and coracoid were larger and more definite structures but still did not closely resemble those of adults. The scapula was an irregularly rectangular bone with a deep notch in its anterior surface; the dorsal surface had not completed ossification. The coracoid was denser towards the cleithrum, but otherwise was much the same as described in younger specimens. Radials were not present (Fig. 1C). The girdle supported paired fins which had six rays each (one specimen of P. albigutta had seven rays on each side).

From the study of the osteology of the heads of the three species of flounders by Beirne (1966), it was reported that in the head area similarities were more striking than differences. However, total numbers of gill rakers and numbers of left dentary teeth were found by her to show significant differences. When these two characters were combined *P. dentatus* was 100% separable from *P. albigutta* and there was a divergence of 76% between *P. lethostigma* and *P. albigutta*. The findings of Beirne notwithstanding, the most easily recognizable differences were in the trunk region.

If the number of vertebrae for each specimen of each species is plotted on the abscissa and the number of anal fin rays of the same specimens are plotted on the ordinate, the species separate into three definite groups. *Paralichthys dentatus* has high vertebral counts and high fin ray counts; *P. lethostigma* has low vertebral counts and high fin ray numbers; and *P. albigutta* has low counts for both characters (Fig. 4). Similar results could be obtained by plotting vertebral counts against dorsal fin ray counts, lateral-line scale counts, or pterygiophore counts.

One of the purposes of the present research was to find if there were additional methods of distinguishing the three species, and combinations of characters are often useful to this end. The results of the current study when considered with that of Beirne (1966) add considerable evidence in support of Ginsburg (1952).

Summary

One hundred-nineteen specimens were used in the study, of which 24 were *P. albigutta* (20 cleared specimens), 51 were *P. lethostigma* (42

cleared), and 44 were P. dentatus (24 cleared). Skeletal elements were compared by observation and measurement, and meristic counts were made. Although most of the osteological characters were held in common by the species, enough differences were found to make positive separation possible.

The most useful methods for determining differences between P. dentatus, P. albigutta and P. lethostigma were as follows:

1. Paralichthys dentatus had the largest number of vertebrae; P. lethostigma and P. albigutta had fewer vertebrae with identical means.

2. Lateral-line scale counts were highest on both sides of the fish in P. dentatus. P. albigutta had the lowest number of scales, and P. lethostigma had an intermediate count. All three species showed significant separation from each other on this character.

3. P. albigutta was widely separated from the other two species in pterygiophore counts. P. dentatus and P. lethostigma had higher counts that approximated each other.

4. Dorsal and anal fin ray counts yielded data similar to that given by the pterygiophores. By means of these characters, P. albigutta with the lowest numbers of rays, was easily separated from P. dentatus and P. lethostigma.

5. When anal fin ray counts were plotted on a graph against vertebral counts for each specimen of each species, 100% separation of all three species was obtained.

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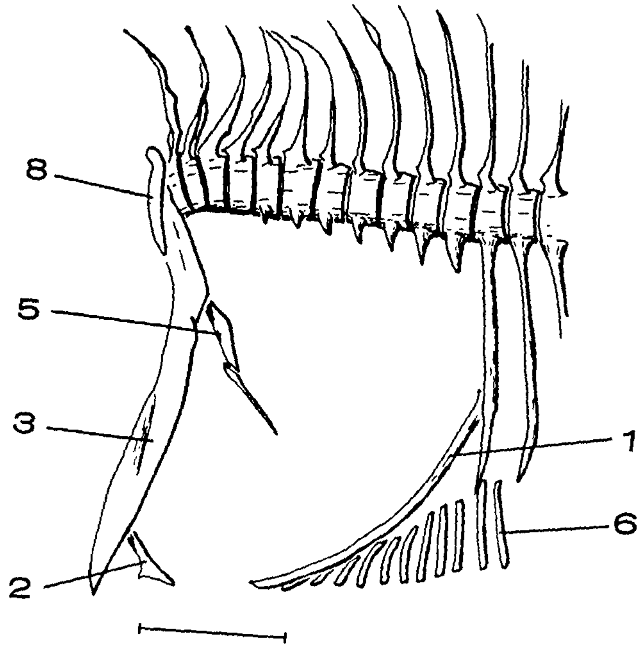
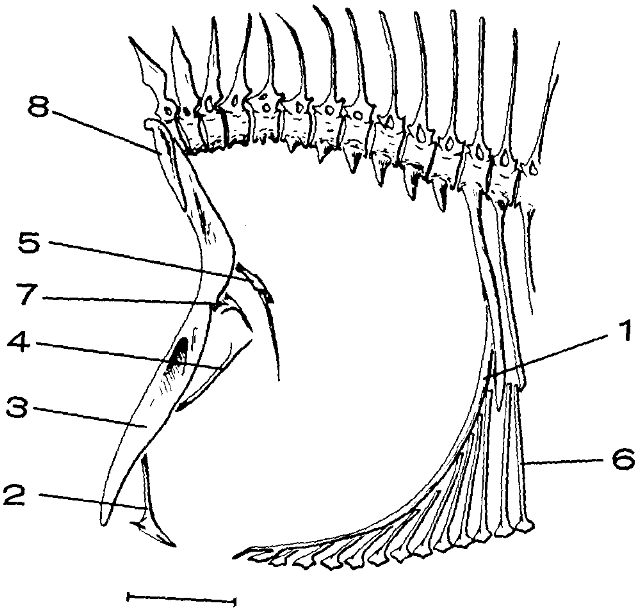
Fig. 1. Composite of the trunk and appendicular skeletons of *Paralichthys albigutta*, *P. dentatus* and *P. lethostigma*.

A. 11 mm st. lg., scale 1 mm. B. 28.1 mm st. lg., scale 2 mm. C. 37.5 mm st. lg., scale 2 mm. 1. Abdominal Rod 2. Basipterygium 3. Cleithrum 4. Coracoid 5. Postcleithrum 6. Pterygiophore 7. Scapula 8. Supracleithrum



A



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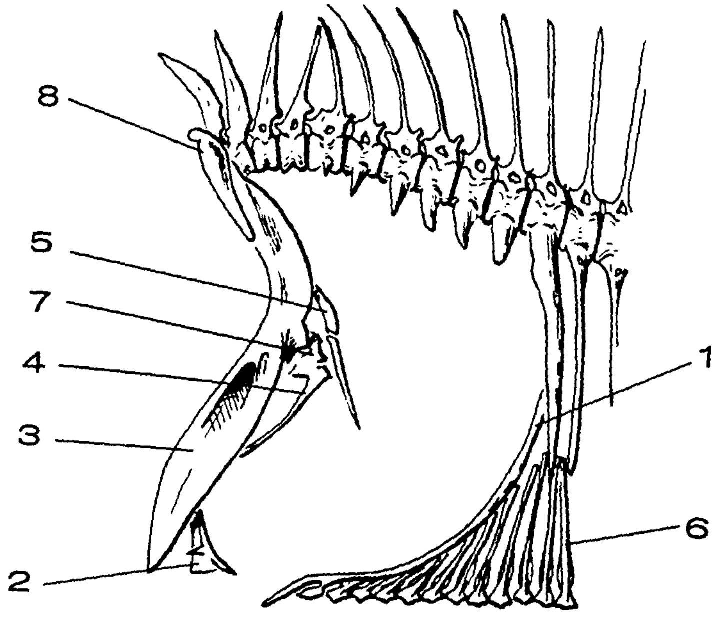


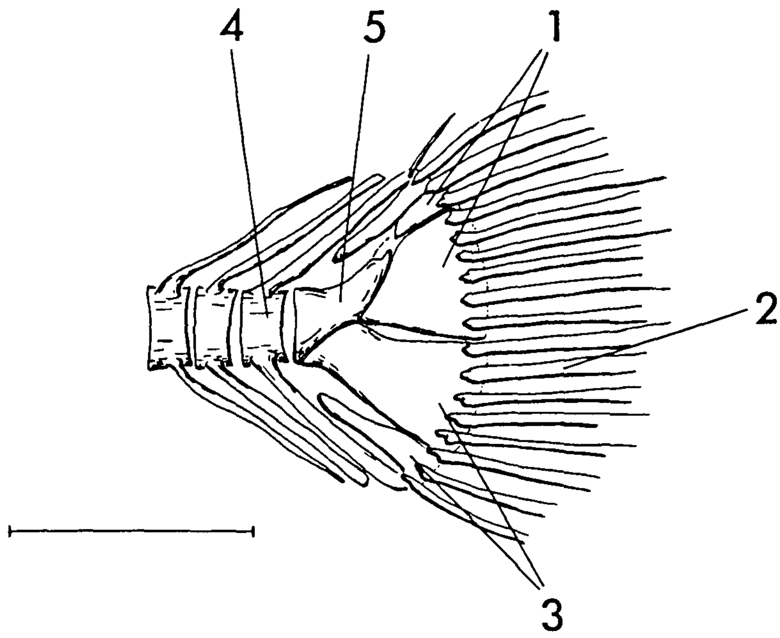
C

Fig. 2. Composite of the caudal skeleton of *Paralichthys albigutta*, *P. dentatus* and *P. lethostigma*.

A. 9.6 mm st. lg., scale 1 mm. B. 27.0 mm st. lg., scale 1 mm.

1. Epurals 2. Fin Rays 3. Hypurals 4. Penultimate Vertebra 5. Urostyle

A



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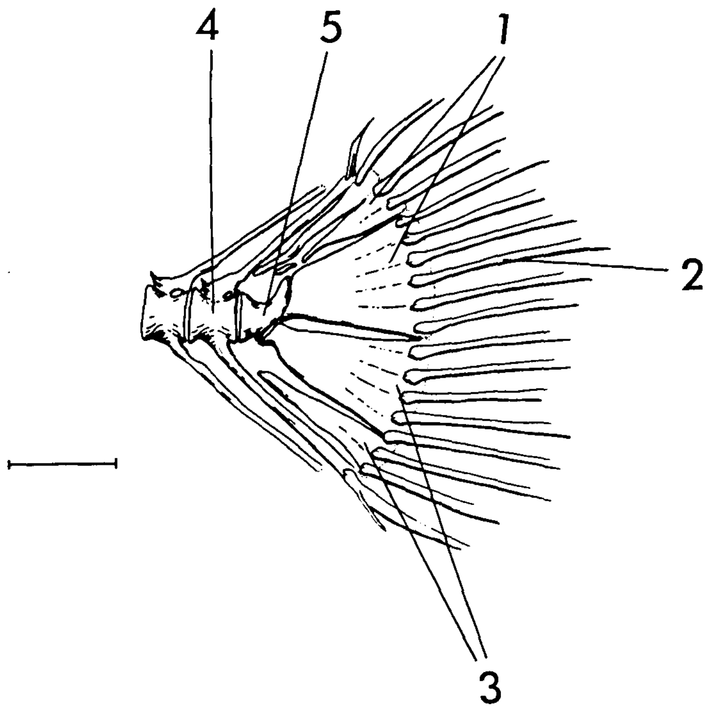
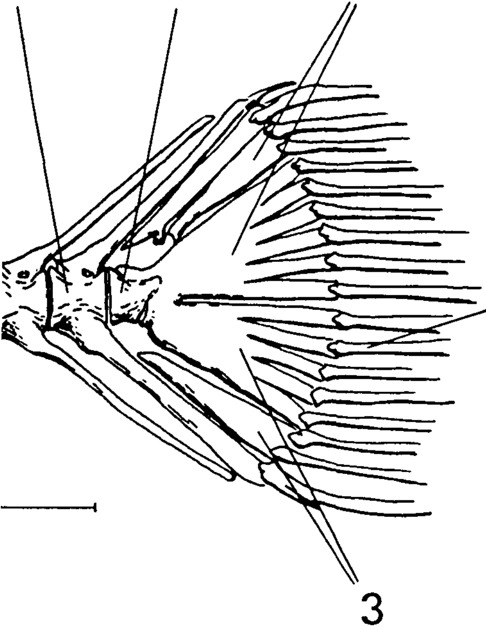


Fig. 3. Composite of the caudal skeletons of *Paralichthys albigutta*, *P. dentatus* and *P. lethostigma*.

A. 37.5 mm st. lg., scale 2 mm. B. 47.0 mm st. lg., scale 2 mm.

1. Epurals 2. Fin Rays 3. Hypurals 4. Penultimate Vertebra 5. Urostyle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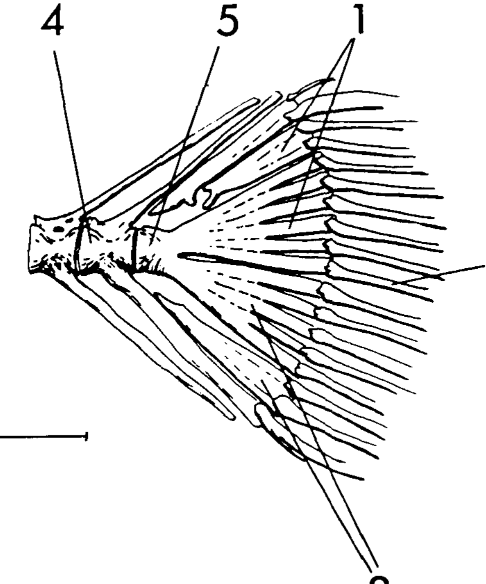
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Fig. 4. Scatter diagram of anal fin ray counts and vertebral counts of *Paralichthys albigutta*, *P. dentatus* and *P. lethostigma*. Numbers beside marked coordinates indicate multiple specimens with identical counts.

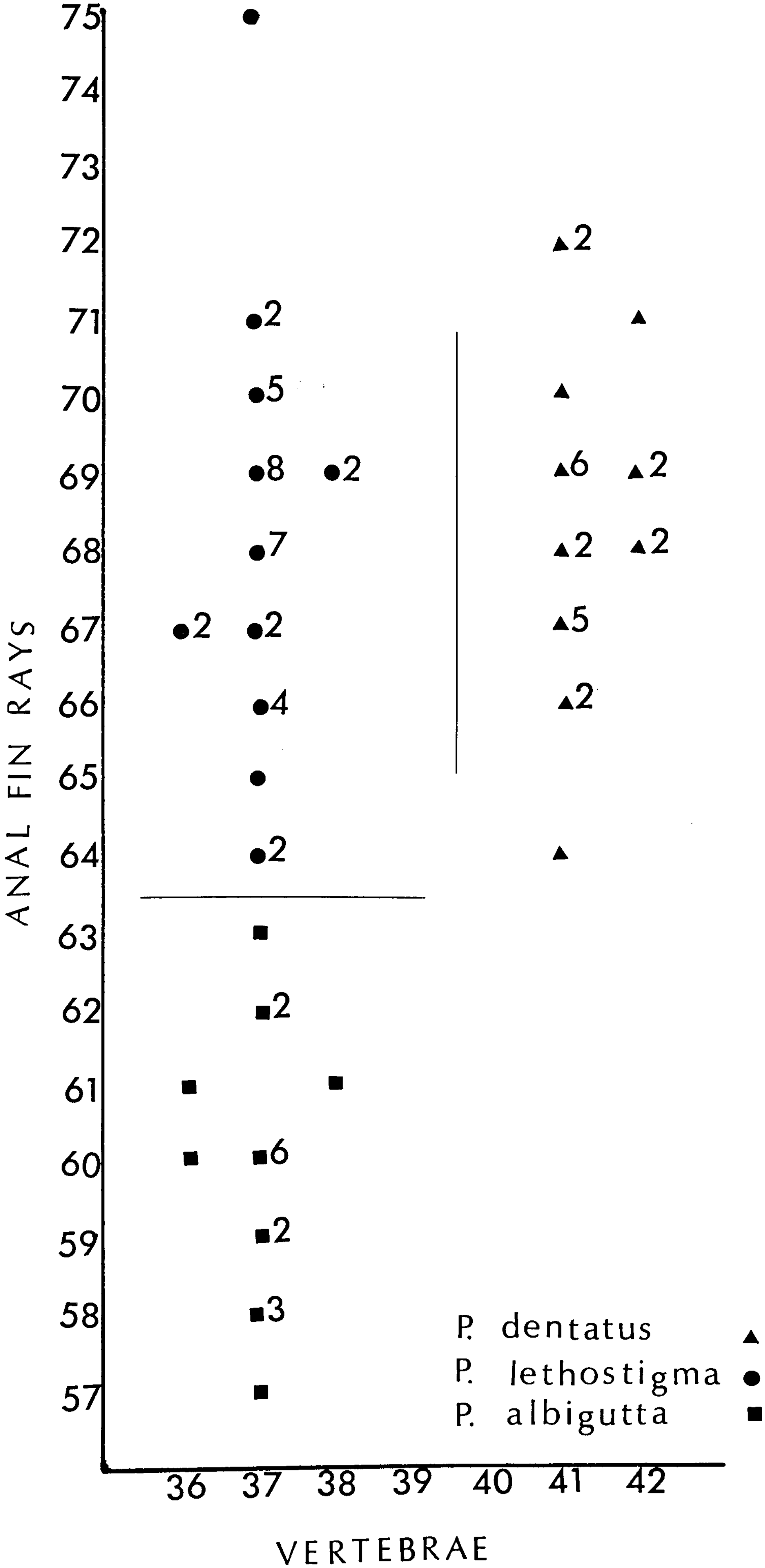
ANAL FIN RAYS
VERTEBRAE
P. dentatus
P. lethostigma
P. albigutta

Table 1. Frequency distribution of lateral-line scales of *Paralichthys dentatus*, *P. lethostigma* and *P. albigutta* from the blind side.

	Lateral-line Scales																																
	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	No.	$\bar{x}$	SD
P. dentatus											1					1	3	4	1	2	4	1	1	2	1	2		1		1	25	77.8	4.16
P. lethostigma						1		1	3	1	2	2	2	3	3			2	1	3											24	70.5	3.98
P. albigutta	1			2	1	4	3	3	2																						16	63.3	2.02

Table 2. Frequency distributions of external meristic characters of _Paralichthys_ _dentatus_, _P._ _lethostigma_ and _P._ _albigutta_ from the eyed side.

A. Scales in Straight Part of Lateral Line

	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	No.	x̄	SD
_P._ _dentatus_																		1		2	4	4	3	2	2	3	1	1	1	1	25	66.5	2.9
_P._ _lethostigma_													1	1	1		5	5	1	3	5	2									24	61.5	2.4
_P._ _albigutta_	1		1	1	1	3	2	1	2	2		1		1																	16	50.3	3.2

B. Dorsal Fin Rays

	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	No.	x̄	SD
_P._ _dentatus_											2	2	4	3	2	4	3	1			21	88.4	2.0
_P._ _lethostigma_							1	1	3	2	4	3	3	4	9	1	3	1		1	36	87.2	3.0
_P._ _albigutta_	2	2	1	4	2	1	3	1													16	78.4	2.1

C. Anal Fin Rays

	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	No.	x̄	SD
_P._ _dentatus_								1		2	5	4	8	1	1	2				24	68.3	1.5
_P._ _lethostigma_								2	1	4	4	7	10	6	2				1	37	68.3	2.0
_P._ _albigutta_	1	3	2	7	2	2	1													18	59.9	1.5

Table 3. Frequency distributions of meristic characters of the trunk skeletons of *Paralichthys dentatus*, *P. lethostigma* and *P. albiguttta* from the eyed side.

A. Vertebrae

	36	37	38	39	40	41	42	No.	$\bar{x}$	SD
P. dentatus						19	5	24	41.2	0.4
P. lethostigma	3	37	2					42	36.9	0.3
P. albiguttta	2	17	1					20	36.9	0.3

B. Dorsal Pterygiophores

	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	No.	$\bar{x}$	SD
P. dentatus													2	2	2	5	4	2	2	1	1		21	87.5	2.1
P. lethostigma									2	2	2	2	5	4	5	3	10	2	2	1		1	41	86.9	2.9
P. albiguttta	1	1	1	2	1	4	2	3	2														17	77.8	2.2

C. Anal Pterygiophores

	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	No.	$\bar{x}$	SD
P. dentatus									1	4	2	3	8	2	1	1	2			24	66.6	2.1
P. lethostigma								1	2	5	4	13	8	6	2				1	42	66.2	1.9
P. albiguttta	2	2	3	6	3	2	1													19	57.8	1.6