TULANE STUDIES
IN ZOOLOGY

Volume 1, Number 12 August 26, 1954

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TULANE UNIVERSITY
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Price for this number: $0.35.

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THE TAXONOMIC STATUS OF THE MID-GULF COAST *AMPHIUMA*[1]

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The identity of the *Amphiuma* of the Gulf Coast has been studied by Ryder (1880), Cope (1886), Brimley (1909), Goin (1938) and Baker (1947). These investigators have drawn different conclusions from the study of similar material. Their conclusions have been based on analyses utilizing either one or several of the following characters: number of toes, color, ratio of total length to body length, number of costal grooves and ratio of body length to limb lengths.

The early investigators, Ryder, Cope and Brimley, had only a small number of individuals available for their studies. The more recent workers, Goin and Baker, considered populations of individuals, but none of the investigators, measured or dealt with ontogenetic variation, allometric growth or sexual dimorphism. Since some authors give only the mean of the ratios and of the costal groove number, it is not possible to evaluate the characters reported. A new approach was required to evaluate the characters previously used and to investigate other differences, if any, between the populations.

Historical—Garden described *Amphiuma means* in 1821. In 1827 Cuvier described a three-toed individual as *Amphiuma tridactylum* and compared it with a two-toed one: "This description of the *Amphiuma* of three digits agrees nearly in everything to the kind with two digits. Its general form is the same; its diameter is one-twentieth of its length; the length of its head is nearly one-fourteenth; its tail is exactly one-fourth. It has similar folds on each side. Its color is the same. Summarizing, it is necessary for descriptive purposes to look closely at its extremities. Then one sees that each of its limbs is divided into three perfectly distinct toes; it is in reality its only exterior character which is readily discernable."

The three-toed individuals were placed in the genus *Mauraenopsis* by Fitzinger (1843). Ryder (1880) studied a series of young *Amphiuma* from Biloxi, Mississippi and reestablished the three-toed salamander in the genus *Amphiuma.* Ryder based his classification solely on the number of digits. He stated "so that in some the number of digits (two) is characteristic of *Amphiuma,* and in others (three) they are characteristic of *Mauraenopsis.*" Cope (1886) could not separate his specimens into two-toed and three-toed groups and so he classified all individuals, regardless of the number of toes, as *Amphiuma means.*

Brimley (1909) called the three-toed animal *Amphiuma tridactylum.* Brimley stated that the three-toed specimens differed from the

[1] From a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science of the Graduate School of Tulane University.

two-toed ones in being lighter in color on both the dorsal and ventral surfaces and in being distinctly bicolored; the limbs of the three-toed *Amphiuma* were "more strongly developed." The character used in separating the two forms, prior to 1909, was number of toes. Brimley also reported that he had never seen a two-toed individual which had attained as great a size as the three-toed ones; one three-toed individual examined by Brimley was 736.6 mm in length.

Goin (1938) examined 206 individuals and found that the costal groove count and the ratio of total length to body length may also be used as taxonomic characters. Considering the region where the two ranges meet, he stated "coloration, costal groove count and ratio of total length to body length change more or less gradually, but the two-toed specimens of the east interdigitate with the three-toed specimens of the Mississippi Valley." According to Goin, *Amphiuma means means* is a two-toed, unicolored animal with 57-60 costal grooves and a ratio of total length to body length which averages 1.33. *Amphiuma means tridactylum* is a three-toed, bicolored animal with 60-64 costal grooves and a ratio of total length to body length which averages 1.29. Goin considered the area of intergradation as the region between Lake Pontchartrain in southeastern Louisiana and Liberty County, Florida.

Baker (1947) examined over 280 *Amphiuma* and concluded that "two species of Amphiumae, *Amphiuma means* and *Amphiuma tridactylum,* occur, each occupying a distinct ecological range." Baker found no evidence of intergrades and stated that all known specimens could be designated as one of these species by the "valid species characters of number of toes, body length in total length, length of limbs in body length and coloration."

Procedures

Number of toes and coloration—In the course of this investigation, 537 *Amphiuma* were examined. Two hundred and forty-six of these were two-toed, two hundred and eighty-eight were three-toed, and three individuals had both two- and three-toed limbs (Table 1). All individuals examined are placed into one of six arbitrary geographical series to determine the degree of difference, if any, between the series. Series 1 includes all three-toed *Amphiuma* from the west bank of the Mississippi River westward. The most western locality of an *Amphiuma* examined in this study is nine miles northwest of Saratoga, Hardin County, Texas. Series 2 consists of the individuals from Audubon Park in New Orleans, Louisiana. Series 3 includes all individuals in the alluvial valley east of the Mississippi River. Included in Series 4 are the three-toed *Amphiuma* from the region east of the alluvial valley of the Mississippi River to western Alabama. This series includes individuals from extreme southeastern Louisiana and Mississippi.

Series 5 consists of the two-toed individuals from the same geographical area as Series 4, and Series 6 consists of those individuals from the Atlantic coastal states westward to Liberty County, Florida

TABLE 1.
NUMBER OF TOES

Series	Number with Two Toes	Number with Uncertain Number of Toes	Number with Three Toes	Total Number Examined
1	0	0	33	33
2	0	0	191	191
3	0	0	17	17
4	0	3	47	133
5	83		0	
6	163	0	0	163
Total	246	3	288	537

Figure 1. Distribution of the 537 *Amphiuma* examined in this research. The circular and X-shaped symbols represent three-toed and two-toed individuals respectively.

(figure 1). The individuals from western Alabama to Liberty County, Florida, were not placed into any of the six series because they were too few in number to constitute an additional series. Series 4 and 5 extend as far east as the locality where both the two-toed and the three-toed individuals are found in close association. This arbitrary grouping into series does not mean to imply that the individuals from western Alabama to Liberty County, Florida, are different from those in Series 4 and 5.

Most of the specimens utilized in this study are deposited in the collections of Tulane University (TU); a few were borrowed from the American Museum of Natural History, the United States National Museum, and the University of Florida.

An external morphological study was made on each individual. The following information was recorded: museum catalogue number, date collected, collector, tail length, body length, hind-limb length, fore-limb length, head length, number of toes, sex and coloration. The length of the tail was taken as the distance from the anterior end of the cloacal opening to the tip of the tail. The limbs were pressed posteriorly and horizontally along the body and the lengths were recorded as the distances from the base of the limb to the distal end of the longest digit. In the three-toed individuals, the longest digit is the middle one and the outermost toe is the smallest (when the limbs are pressed posteriorly and horizontally along the body). The measurements were made consistently on the right limbs of the animals unless the right limbs were mutilated. The head length was taken as the straight line distance from the anterior end of the brachial fissure to the tip of the nose. The number of toes on each limb was recorded in a formula arrangement, *e.g.* 2/2 2/2.

The formula is read from left to right, and the numbers represent, respectively, the number of toes on the right fore-limb, the number of toes on the left fore-limb, the number of toes on the right hind-limb and the number of toes on the left hind-limb. If the individuals were sexed, this was done by dissection. The dorsal, ventral and throat colorations were recorded. Table 2 indicates the throat coloration of the specimens from the six geographical series.

The measurements were made with vernier calipers, except when the structure was greater than 125 mm. In the latter case or if the preserved animal were coiled, a string was used and transferred to a ruler calibrated in millimeters. The measurements were recorded to the nearest millimeter. All of the measurements were made on preserved specimens. There is undoubtedly some differential shrinkage between the various body parts, resulting from the preservation, but this possible error is likely to be minimized since all of the individuals examined were preserved by comparable methods.

Only those individuals were measured whose parts were not mutilated. Mutilation could usually be detected by the presence of scar tissue. Frequently, *Amphiuma* have part of a limb or an entire limb missing. Obviously, it is easier to detect a mutilation which has

TABLE 2.
THROAT COLORATION

Series	Number without Throat Patch	Number with Uncertain Throat Color	Number with Throat Patch	Total Number Examined
1	0	0	33	33
2	0	0	191	191
3	0	0	17	17
4	0	3	47	133
5	83		0	
6	163	0	0	163
Total	246	3	288	537

occurred recently in the life of an animal than it is to determine one which occurred very early in the life of the individual. It is unusual to find all four limbs of an animal mutilated. The hind-limbs are normally longer than the fore-limbs, except in individuals less than a hundred millimeters in length. One can usually confirm the opinion of whether or not a digital mutilation has occurred by comparing the measurements of all four limbs.

Body proportions—Heretofore, three assumptions have been made by some workers who have considered body proportions as taxonomic characters in *Amphiuma.* These assumptions include absence of sexual dimorphism, high correlation between the dimensions of the body parts, no ontogenetic variation. The latter is illustrated by Baker's use of simple ratios, implying that body proportions do not change with an increase in age (body length).

If these assumptions represent the facts, then the previous work is valid, but if one or more of the assumptions are incorrect, the results may require some qualification. Statistical methods can often be used to give quantitative estimates of these assumptions. The preliminary procedure was to determine the presence or absence of sexual dimorphism, the degree of correlation between the body parts, and whether or not ontogenetic variation was present.

To determine the presence or absence of sexual dimorphism, four groups of graphs were set up, each group being composed of six graphs representing the six geographical series. The four groups represent the following four body proportions: head length to body length, fore-limb length to body length, hind-limb length to body length and tail length to body length. For each graph the horizontal scale represents the body length in millimeters and the vertical scale represents the other body part. The presence or absence of important sexual dimorphism can be estimated by inspection. An inspection of the scattergrams indicates that the males and females do not fall

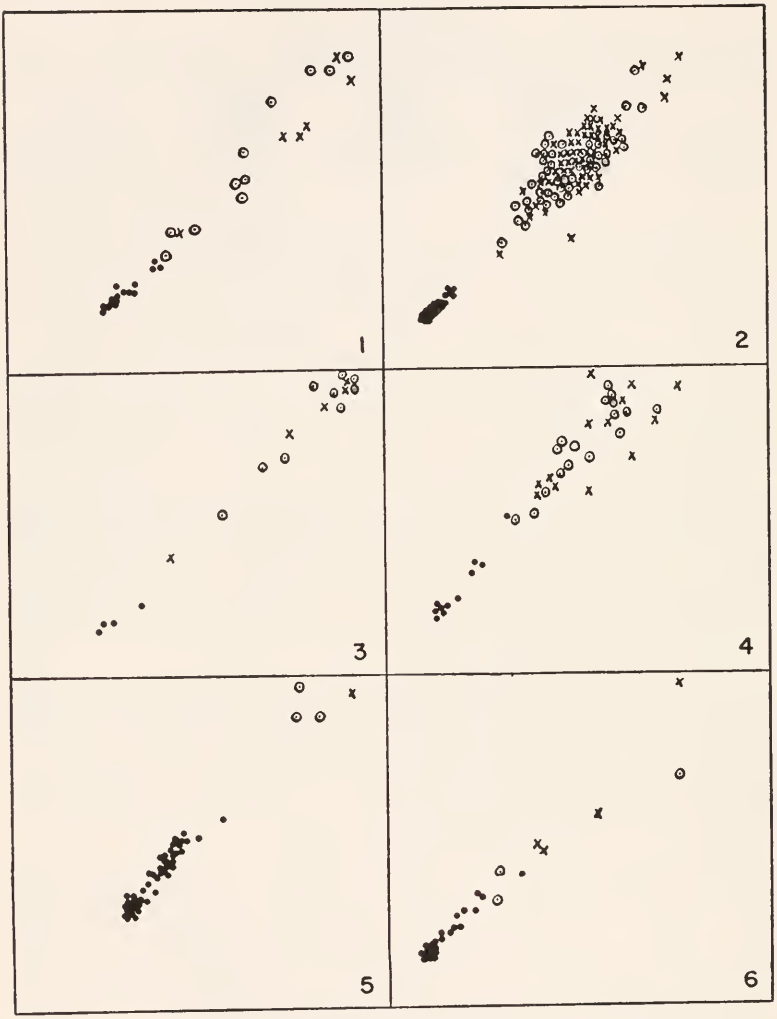

Figure 2. Scattergrams showing the relationship of head length to body length for each of the six series. The three symbols, X, circle with dot and plain dot, identify respectively male, female and unsexed individuals.

into two separate groups in any of the series; therefore, if sexual dimorphism exists it is negligible (figures 2-5).

Obviously, there is a definite relationship of head length, fore-limb length, hind-limb length and tail length to body length so the problem is concerned with the degree of correlation and the nature of the correlation (*e.g.,* simple and linear). The degree of correlation of

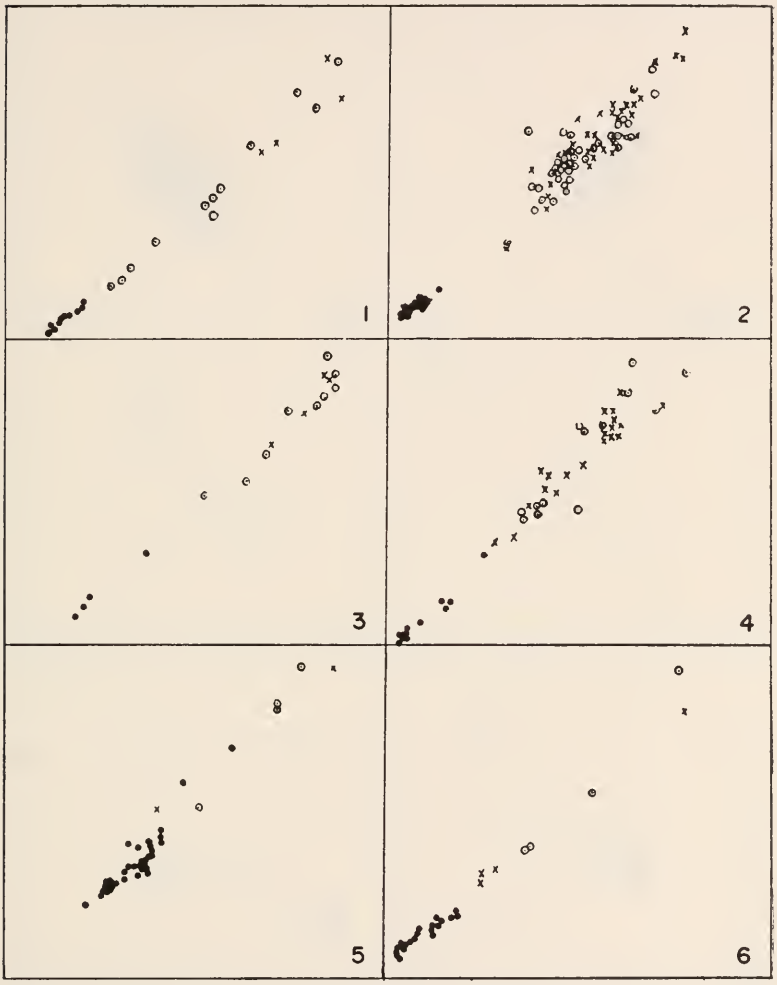

Figure 3. Scattergrams showing the relationship of fore-limb length to body length for each of the six series. Symbols as in fig. 2.

the various body parts with the body length was determined by calculating the correlation coefficients (Tables 3-6). The method used was Pearson's product-moment method using grouped data cross-tabulated. Twenty-four correlation tables were constructed. In constructing the correlation tables, the body length was considered the independent variable and the other body part length the dependent variable (Peatman, 1947).

Ontogenetic variation—To determine whether or not body pro-

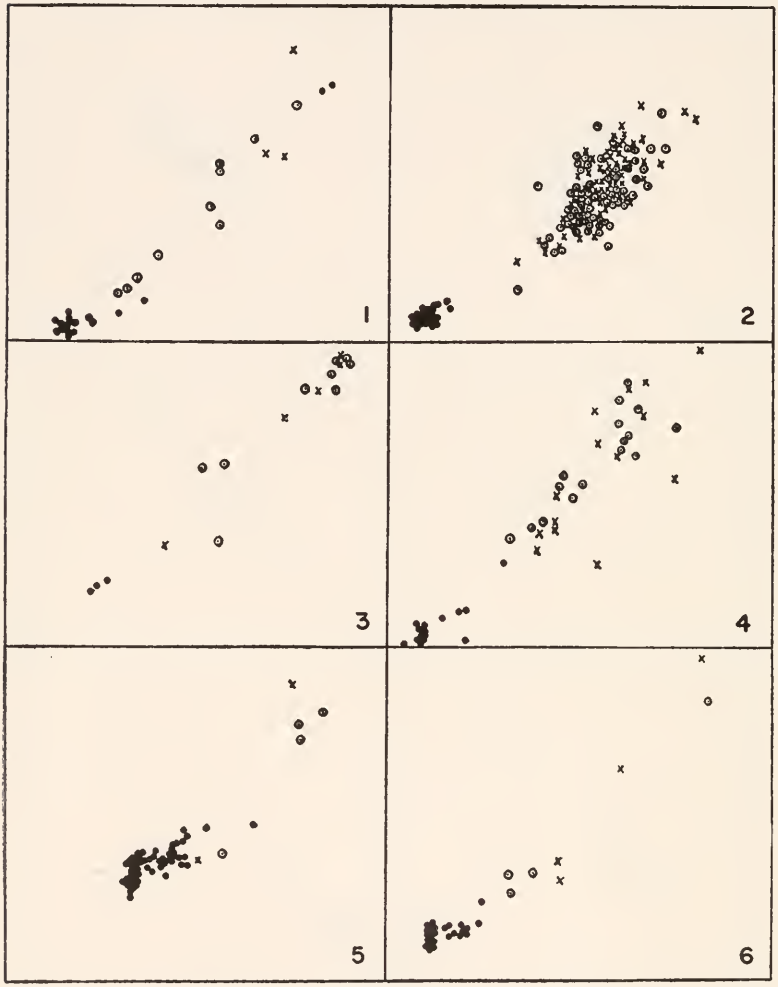

Figure 4. Scattergrams showing the relationship of hind-limb length to bo dylength for each of the six series. Symbols as in fig. 2.

portions remain constant as the animal increases in age (length), the regression lines for the four body parts of each of the six series were calculated. The correlations in ontogenetic series are nearly always high, but inspection of the scattergrams indicate that linearity may be assumed for practical purposes.

The fact that the line is linear for the four body proportions of each of the six series was established by the product-moment correlation coefficients. Thus, the regression equation for the straight line is:

$$X = a + aB$$

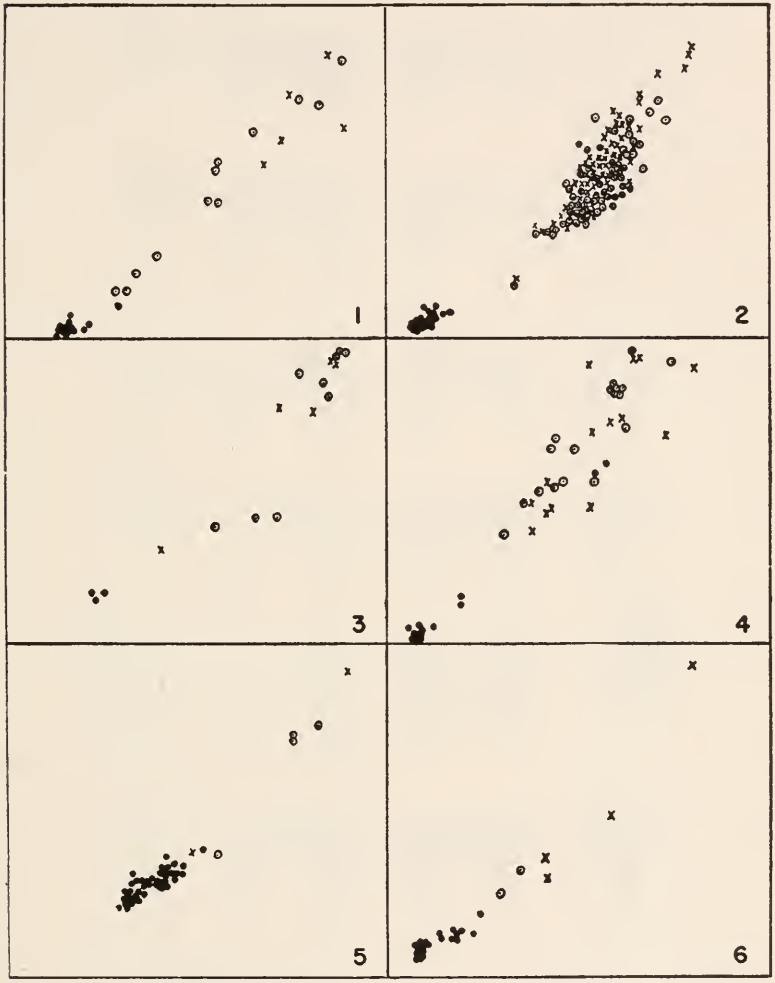

Figure 5. Scattergrams showing the relationship of tail length to body length for each of the six series. Symbols as in fig. 2.

where X is equal to either head length, fore-limb length, hind-limb length or tail length; a and b are the regression constants and B is the body length corresponding to a particular X length. In order to determine X for any particular body length, B, the regression constants must be calculated.

The three methods suggested by Klauber (1943) for calculating the regression constants are: (1) analytical methods, (2) method of least squares and (3) the graphical method. Of these, the latter was

TABLE 3.
RELATIONSHIP OF HEAD LENGTH TO BODY LENGTH

Series	1	2	3	4	5	6
Total number of individuals	33	191	17	47	84	163
Body length range, mm	63.5-566.8	43.2-535	51.5-504.5	73.1-563.3	63-442.1	45.1-600
Correlation coefficient, r	0.981	0.936	0.985	0.968	0.919	0.989
	± 0.0044	± 0.0061	± 0.020	± 0.0063	± 0.011	± 0.0012
Regression constants	a 0.82	a 1.034	a 0.262	a 0.605	a 1.650	a 1.830
	b 0.125	b 0.119	b 0.116	b 0.109	b 0.096	b 0.090
Restricted number of specimens	13	68	12	21	5	8
Body length range, mm	330.6-566.8	350.8-535	341.6-504.1	353.3-563.3	285-442.1	249.1-600
Standard body length, mm	400	400	400	400	400	400
Standardized head length range, mm	39.3-59.7	35.6-51.2	40.5-51.4	34.3-52.8	40.1-45.8	38.5-49.5
Mean head length equated to standard body length, mm	47.54	43.1	44.8	45.02	44.0	41.3
Standard error of mean, mm	± 1.38	± 0.233	± 0.92	± 0.86	± 0.95	± 1.40
Standard deviation, mm	4.97	4.00	3.05	3.86	1.90	3.71

TABLE 4.
RELATIONSHIP OF FORE-LIMB LENGTH TO BODY LENGTH

Series	1	2	3	4	5	6
Total number of individuals	33	191	17	47	84	163
Body length range, mm	63.5-566.8	43.2-535	51.5-504.5	73.1-563.3	63-442.1	45.1-600
Correlation coefficient, r	0.962	0.919	0.595	0.929	0.901	0.968
	± 0.089	± 0.0074	± 0.104	± 0.013	± 0.017	± 0.0034
Regression constants	a 1.404	a 1.68	a 1.72	a 0.329	a 0.526	a 1.34
	b 0.0234	b 0.0297	b 0.0261	b 0.0276	b 0.021	b 0.0207
Restricted number of specimens	13	68	12	21	5	8
Body length range, mm	330.6-566.8	350.8-535	341.6-504.1	353.3-563.3	285-422.1	249.1-600
Standard body length, mm	400	400	400	400	400	400
Standardized limb length range, mm	9.3-14.2	9.1-13.5	10.1-13.0	7.0-13.2	6.2-8.9	6.8-10.9
Mean fore limb length equated to standard body length, mm	10.90	10.90	12.09	11.25	7.99	8.50
Standard error of mean, mm	± 0.257	± 0.164	± 0.250	± 0.427	± 0.121	± 0.576
Standard deviation, mm	0.894	1.35	0.83	1.86	0.767	1.41

TABLE 5.
RELATIONSHIP OF HIND-LIMB LENGTH TO BODY LENGTH

Series	1	2	3	4	5	6
Total number of individuals	33	191	17	47	84	163
Body length range, mm	63.5-566.8	43.2-535	51.5-504.5	73.1-563.3	63-442.1	45.1-600
Correlation coefficient, r	0.979	0.943	0.926	0.952	0.909	0.949
	± 0.0049	± 0.0055	± 0.036	± 0.0093	± 0.013	± 0.0078
Regression constants	a 0.18	a 1.033	a 0.72	a 1.224	a 0.0351	a 1.572
	b 0.0422	b 0.0417	b 0.0431	b 0.0368	b 0.0319	b 0.0237
Restricted number of specimens	13	68	12	21	5	8
Body length range, mm	330.6-566.8	350.8-535	341.6-504.1	353.3-563.3	285-442.1	249.1-600
Standard body length, mm	400	400	400	400	400	400
Standardized limb length, range, mm	15.2-18.9	13.1-20.1	14.7-18.8	11.8-23.8	9.8-14.2	9.5-14.0
Mean hind limb length equated to standard body length, mm	17.27	16.49	18.38	17.35	12.75	11.70
Standard error of mean, mm	± 0.148	± 0.034	± 0.139	± 0.445	± 0.316	± 0.596
Standard deviation, mm	1.33	1.53	1.24	2.91	1.26	1.73

TABLE 6.
RELATION OF TAIL LENGTH TO BODY LENGTH

Series	1	2	3	4	5	6
Total number of specimens ..	33	191	17	47	84	163
Body length range, mm	63.5-566.8	43.2-535	51.5-504.1	73.1-563.3	63-442.1	45.1-588.3
Correlation coefficient, r	0.924	0.998	0.981	0.931	0.960	0.860
	± 0.017	± 0.00020	± 0.0062	± 0.013	± 0.0057	± 0.014
Regression constants	a 2.976	a 4.005	a 3.173	a 4.209	a 2.775	a 5.53
	b 0.338	b 0.343	b 0.353	b 0.362	b 0.380	b 0.389
Restricted number of specimens	13	68	12	21	5	8
Body length range, mm	330.6-566.8	350.8-535	341.6-504.1	353.3-563.3	285-442.1	294.3-588.3
Standard body length, mm ..	400	400	400	400	400	400
Standardized tail length range, mm	112.8-166.6	99.8-149.8	127.9-160.0	105.5-187.4	132.4-169.0	129.7-170.2
Mean tail length equated to standard body length, mm	138.5	127.05	140.2	137.2	145.9	148.95
Standard error of mean, mm	± 4.42	± 1.48	± 2.32	± 4.20	± 4.10	± 5.78
Standard deviation, mm	15.30	12.17	7.70	18.70	8.20	14.16

chosen, the reason being that the same graphs used to determine sexual dimorphism could be used to determine the regression constants.

After obtaininng *a* and *b* for each of the four body proportions for each of the six series, these constants were substituted in their respective regression equations.

From a study of the regression lines, it was determined that the body proportions do not remain constant throughout life. The reason for the foregoing statement is that *a* is not equal to zero (the regression line does not pass through the origin) (Klauber, 1943). However, it should be mentioned that in some cases *a* is almost equal to zero; although, additional collections may or may not reduce *a* to zero. If the proportion remains constant throughout life, the relationship of the head length to the body length should be the same in the adults as it is in the juveniles. If a juvenile with a body length of 50 mm is substituted in the regression equation ($H = 1.034 + 0.119B$), the value of *H* is equal to 6.984 mm. If an adult body length of 600 mm is substituted in the regression equation, *H* is equal to 72.434 mm. The juvenile head length is 14 percent of its body length; the adult head length is only 12 percent of its body length. This indicates that the juveniles have proportionately larger heads than the adults; therefore, the proportionality does not remain constant with an increase in body length.

Standardization—Since there were insufficient numbers of individuals of a single body length in each series, a second method suggested by Klauber was utilized to eliminate ontogenetic variation. This method requires the conversion of the head length, fore-limb length, hind-limb length, and tail length of the individual to a standard body length. In using this method, the assumption was made that any specimen in growing to (or returning to) the standard body length does so by maintaining a constant percentage deviation from the regression line (Klauber, 1941). The standard body length was arbitrarily chosen as 400 mm. In determining the standard body size, the writer was guided by the known biology of *Amphiuma* so that the chosen body length would be within the adult (sexually mature) body length range of the individuals of each of the six series. The females of the Audubon Park population become sexually mature when they attain a body length of 330 mm but the individuals of the Audubon Park population do not attain a size as large as that of *Amphiuma* collected from the surrounding areas (Cagle, 1948). The 400 mm body length should include the sexually mature individuals from all geographical series.

The body length range for the restricted number of specimens was chosen to include the greatest number of individuals, the limiting factor being the exclusion of individiuals less than 249 mm in body length or greater than 600 mm in body length (Tables 3-6). The statistical accuracy sacrificed by limiting the study to fewer specimens (by taking a narrow range) is more than compensated for by the elimination of possible errors in translating the specimens

over too great a distance along their regression lines (Klauber, 1938).

The actual measurements of the body parts of each individual within the restricted ranges were converted to the measurement they would probably have at 400 mm body length by substituting the actual measurements in a standardization formula (Klauber, 1941).

A survey of the available material indicated that a moderate number of individuals (all sizes included) were available in Series 1 through 4, but the number in the restricted size range of Series 5 and 6 was very small. The range could not be widened because the millimeter spread on either side of the mid-point could not exceed 200 mm (Klauber, 1938). Klauber has used as few as five individuals in his snake studies.

In order to determine the standardized body parts for each individual of each series, the individuals from the restricted ranges of Series 1 through 6 were standardized by substituting the actual length of the part and the body length in a standardization formula. The estimated head length that the *Amphiuma* would have at the standard body length of 400 mm is 34.33 mm. Standardized lengths of the body parts, computed for the restricted number of specimens, were gathered into an array (separate for each geographical series) and their statistics computed (Tables 3-6).

Graphical relationships—Bar graphs were constructed to show the relationship of the range, standardized mean, standard error of the mean and the standard deviation of the four body parts for each of the six series. The horizontal axis was calibrated to include the range of the standardized measurements. For each series (geographical locality) the range of variation is shown by a horizontal line and the mean (M) by a short vertical line. The blackened part of each bar comprises two standard errors of the mean ($2\sigma_M$) on either side of M. One-half of each black bar plus the white bar at either end outline one standard deviation (σ) on either side of M. Standard deviation indicates dispersion and two standard errors of the mean indicates reliability (Hubbs and Hubbs, 1953).

Results

Number of toes and coloration—The division of the 537 individuals into the six geographical series shows the Audubon Park individuals (Series 2) to be the most numerically representative of any of the series. Series 6, 5, 4, 1, and 3 follow, respectively, in decreasing numbers. The division of the individuals into size groups of 50 mm to determine the adequacy of the various age groups of each series, yielded the following information: of the three-toed groups, Series 1 was not represented in the size groups of less than 50 mm, of 250-300 mm, and of 350-400 mm; Series 2 was not represented in the size groups of 100-150 mm and 550-600 mm; Series 3 was not represented in the size groups of less than 50 mm, 100-150 mm, 200-250 mm, and 550-600 mm; Series 4 was not represented in the size group of less than 50 mm. The groups not represented

in the two-toed populations by Series 5 are size groups of less than 50 mm, 300-350 mm, 450-500 mm, 500-550 mm, 550-600 mm; by Series 6, 300-350 mm, 450-500 mm, and 500-550 mm (figures 6 and 7).

An inspection of the map showing the geographical distribution of the individuals examined in this research indicates that the two-toed and three-toed individuals occur together in the region where the alluvial valley of the Mississippi River meets the East Gulf Coastal Plain (figure 1). Within this region the proximity of the three-toed *Amphiuma* to the two-toed *Amphiuma* varies. A collection made in Amite, Louisiana, June 8, 1948, consisted of 42 *Amphiuma* taken from the same mud hole; of these, forty-one individuals had two toes on all four limbs and one individual had three toes on all four limbs. There is reason to believe that these individuals are from the same brood. All except one individual had a body length of approximately 214 mm. In the same region individuals with two toes on all four limbs and individuals with three toes on all four limbs have been collected together, either in the same drainage ditches, in adjacent drainage ditches or within a few miles (less

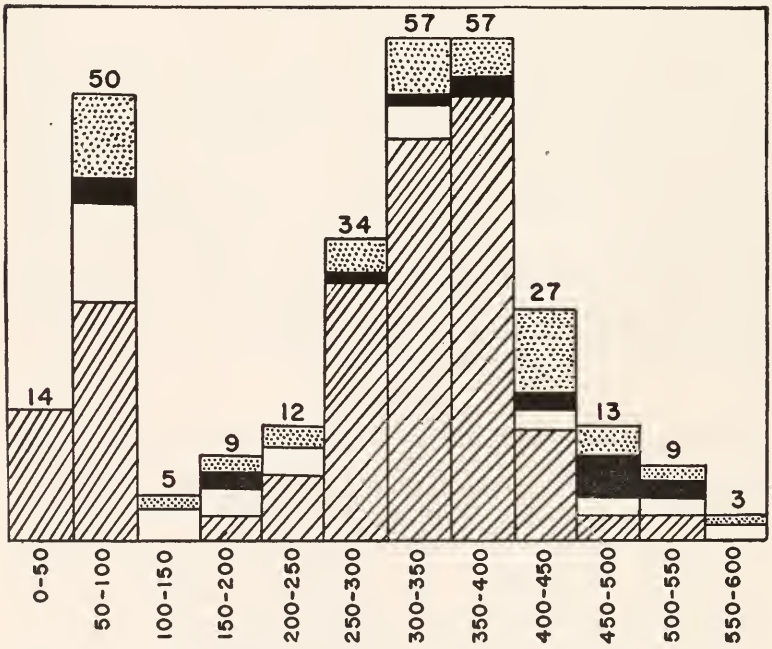

Figure 6. The distribution of body lengths of 290 specimens: Audubon Park (cross-hatched), west of the Mississippi River (clear), eastern alluvial valley (shaded), and Series 4 (stippled). The total number of individuals composing each 50 mm size group is given at the top of each bar of the histogram.

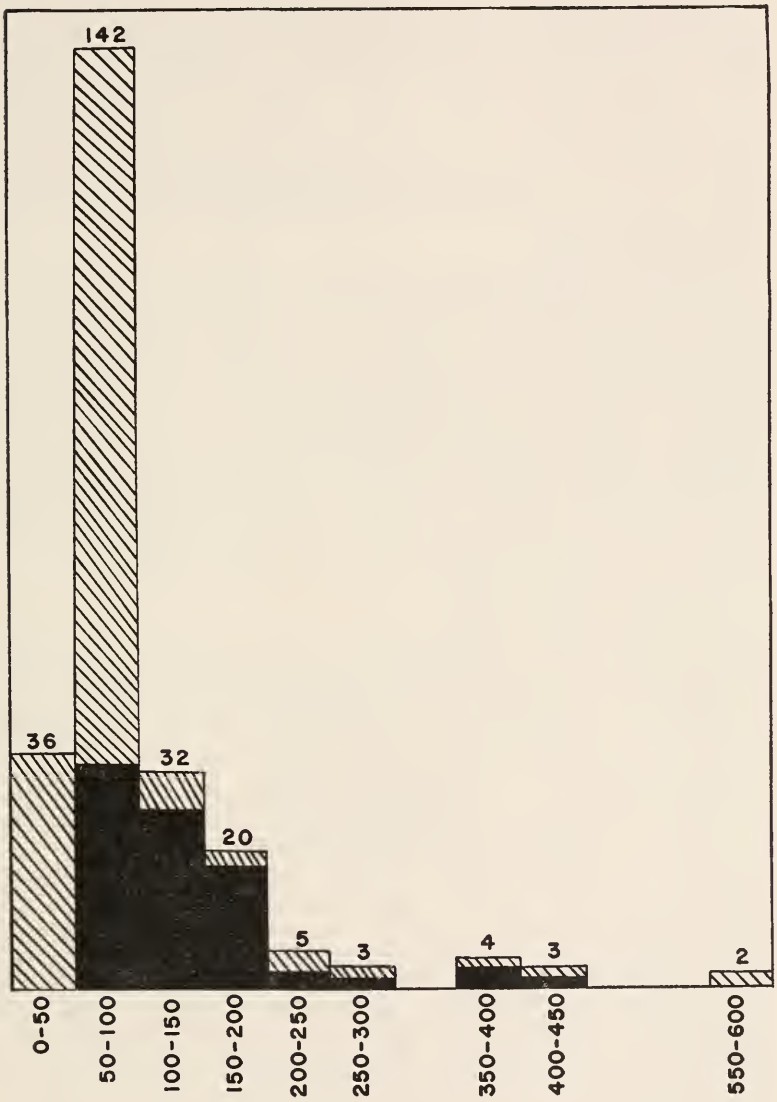

Figure 7. The distribution of body lengths of 83 specimens from Series 5 (shaded) and 163 specimens from the Atlantic coastal states (cross-hatched). The number of individuals comprising each 50 mm size group is given at the top of each bar of the histogram.

than five) of each other.

Three individuals with both two-toed and three-toed limbs on the same individual have been collected within this same geographical area. One of these was collected in Mandeville, Louisiana, in August, 1924, and the other two individuals were collected three miles north of the town of Pearl River, Louisiana. Both of these individuals were collected within the critical area (Series 4 and 5). The one from Mandeville (TU 2207) has a body length of 235.5 mm. The ones collected north of Pearl River in January, 1949 (TU 4777), and January, 1950 (TU 11414), have body lengths of 356 mm and 433 mm respectively.

Courtship activity between a two-toed and a three-toed *Amphiuma* has never been reported. The males of the Audubon Park series are reproductively active from mid-January to mid-May (Cagle, 1948). It is possible that in the region where the alluvial valley of the Mississippi River meets the East Gulf Coastal Plain, the two-toed and three-toed *Amphiuma* are reproductively active at the same time. The reason for the foregoing statement is that a two-toed male *Amphiuma* collected one mile north of Covington, Louisiana, March 6, 1950, was in a reproductive condition. The cloaca of this male was red, swollen and partially everted. The animal had more than ten well defined teeth prints and cuts on its body. These teeth marks and cuts may indicate fighting between the males during the reproductive season (Cagle, 1948).

All individuals examined could be placed into one of the two groups on the basis of number of toes except the three peculiar individuals, or 0.56 percent of the population. The toe formula for the individual from Mandeville is 2/2 3/3; for the two Pearl River specimens, the formulae are 2/2 3/2 and 3/2 2/3. All individuals of the six geographical series can be definitely separated into two-toed groups and three-toed groups on the basis of color, with the exception of the same three individuals which cannot be separated into one of two groups on the basis of the number of toes.

Within the three-toed group there are various degrees of ventral pigmentation, but the overall coloration is never so dark as that of the two-toed individuals. Whether this variation in the ventral coloration within the three-toed group is due to the effects of preservation, ecology, or inheritance has not been determined. The percentage of individuals of two of the three-toed series with ventral pigmentation is as follows: 7.8 percent for the Audubon Park individuals and 19.1 percent for those examined in Series 4. The individuals from the eastern alluvial valley usually have a mottled ventral surface.

Characteristically, the three-toed individuals have a black throat patch. The two-toed individual has no throat patch. Two of the three individuals, which cannot be separated into either the two-toed or the three-toed groups on the basis of a consistant number of toes and coloration, do not have a characteristic black throat patch, but

one of the individuals does have an indication of a throat patch.

Body proportions—Although the ontogenetic spread is comparatively satisfactory for the six series, the frequencies of all size groups should be increased, with the possible exceptions of the eastern two-toed juveniles (50-100 mm) and the Audubon Park adults (300-400 mm).

The correlation coefficients (r) are all high except that for forelimb length to body length of Series 3 (0.595). No explanation is attempted for this low correlation coefficient.

The values for standard deviation (σ) are calculated on the assumption of normal distribution which is not likely to be realized in small samples. There are reasons for distrusting the reliability of the difference that is indicated by the graphical method when the samples include fewer than thirty specimens. Consequently, the limitation of the graphs (figures 8-11) is realized. The graphical analysis indicates why a broad overlap of the dark bars indicates low reliability of the observed difference between two samples and why any considerable separation of these bars indictates a high reliability. The plotting of one standard deviation on either side of the mean indicates an 84 percent separation.

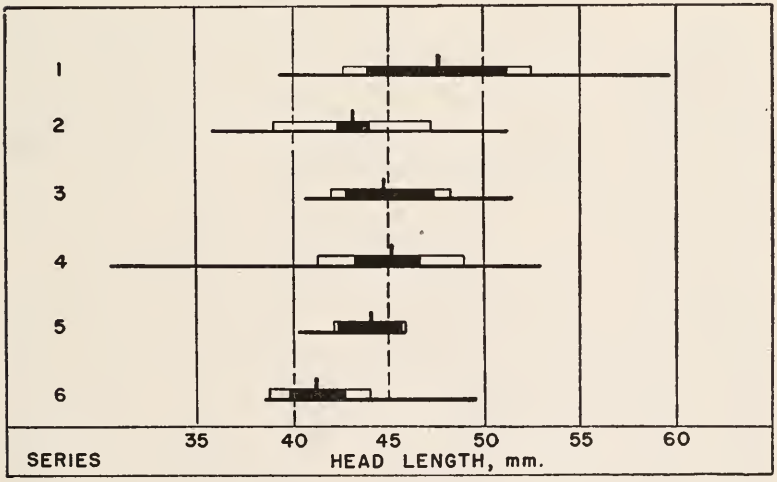

Figure 8. Bar graphs showing the relationship of the range, mean, standard deviation and standard error of the mean for head length for each of the six series. For each locality the range of variation is shown by a horizontal line; the mean (M), by a short vertical line. The blackened part of each bar comprises 2 standard errors of the mean (2 σ M) on either side of M. One-half of each bar plus the white bar at either end outline 1 standard deviation (σ) on either side of M.

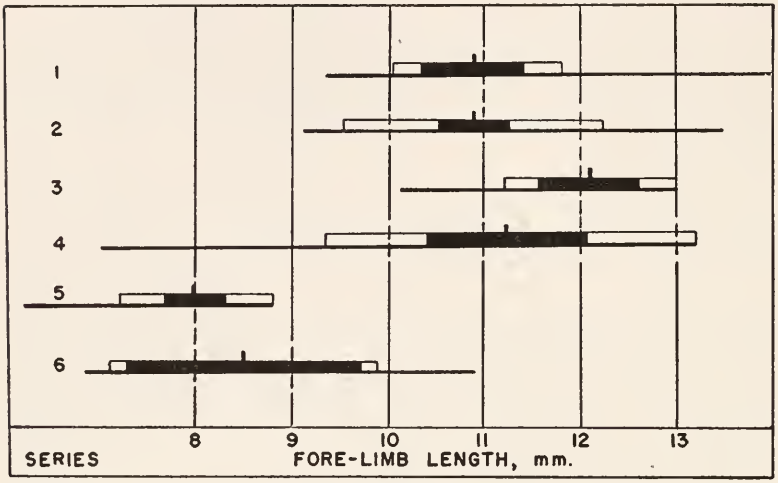

Figure 9. Bar graphs showing the relationship of the range, mean, standard deviation and standard error of the mean for fore-limb length. Arrangement as in fig. 8.

Discussions and Conclusions

Number of toes and coloration—The separation of *Amphiuma* into three groups on the basis of number of toes and coloration gives rise to the question as to whether or not the smallest of the three groups, comprising 0.56 percent of the population, is an intergrade group. If the coloration of these three individuals is due to either ecology or to methods of preserving the animals, and if the variation in the number of toes is due to either mutilation or to the inheritance of the monodactyly or polydactyly type, then *Amphiuma* can be completely separated into two groups, indicating no intergradation.

Since other individuals occurring within the same ecological area do not have the same coloration as these three individuals, indicates that ecology *per se* is not the causative factor in producing this coloration, unless the ecological factor producing it is extremely localized. The latter assumption is unlikely; for, if *Amphiuma* is so susceptible to these extremely localized conditions, a greater degree of natural variation would be expected among the remaining individuals than has been found. It is interesting that the type of coloration shown by these three individuals, which may be a blending resulting from the interbreeding of a bicolored individual with a unicolored individual, is found only in the region where the ranges of the two-toed and the three-toed individuals meet and overlap.

The fact that these three animals have no apparent scar tissue and that the measurements of the left limbs are the same as those of their corresponding right limbs constitutes evidence against the occurrence of mutilation. It is true that monodactyly and polydactyly

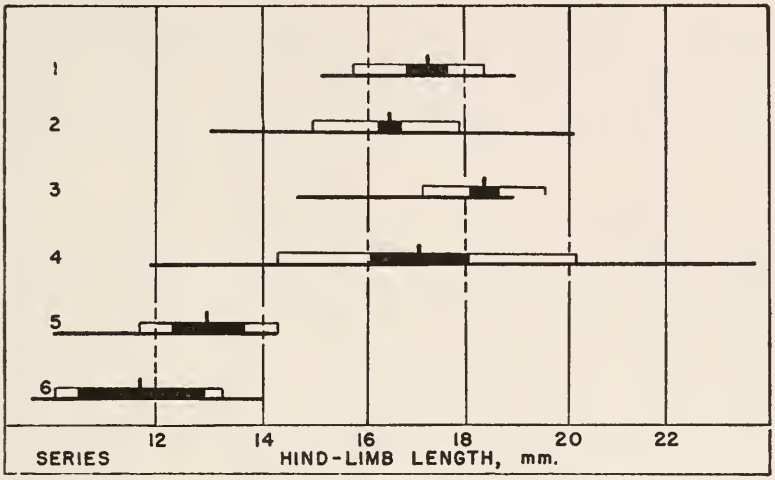

Figure 10. Bar graphs showing the relationship of the range, mean, standard deviation and standard error of the mean for hind-limb length. Arrangement as in fig. 8.

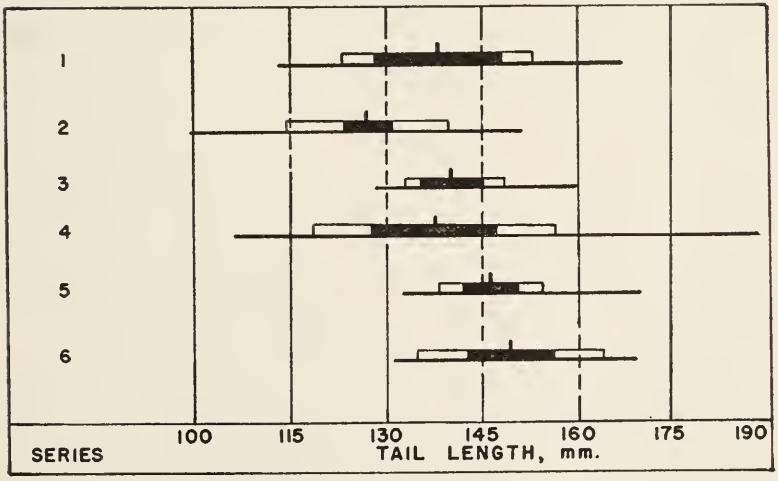

Figure 11. Bar graphs showing the relationship of the range, mean, standard deviation and standard error of the mean for tail length. Arrangement as in fig. 8.

occur in *Amphiuma,* but it is not evident that either one of these conditions expresses itself in any of the three individuals. Polydactyly has been observed only in individuals in the three-toed series, which series were designated by number of toes, coloration (general body color and the presence of throat patch), and geographical location. TU 4777 would have to be considered a three-toed individual because of the presence of the throat patch. The toe formula 2/2 2/3 must then be a result of inheritance (didactyly) as there is no evidence of mutilation. TU 2207 and TU 11414 because of the absence of a throat patch would be considered two-toed individuals. The toe formulae, 2/2 3/3 and 3/2 2/3, respectively, for TU 2207 and TU 11414 must be explained also by inheritance (polydactyly); however, polydactyly has not been observed in two-toed individuals (concerning geographical ranges) in this collection. In spite of the assumption of the presence of polydactyly, and the fact that the throat patch separates the three individuals into two groups, the uniform coloration of the three individuals and the almost identical general coloration of two of the individuals, TU 4777 with the throat patch and TU 11414 without the throat patch, prevents these three individuals from being separated into one of two groups.

Basically, the taxonomic position of the Mid-Gulf Coast *Amphiuma* resolves itself into the taxonomic position of these three individuals; that is, whether or not the three can be separated into one of two groups, a two-toed or a three-toed group, or whether all three should be placed into a third group, an intergrade group. The question is whether these three intermediates are true subspecies or hybrids.

With the exception of three specimens, all two-toed specimens have one toe pattern and all three-toed specimens have another. Since the two tables (1 and 2) are generally concordant and the forms (consistent with respect to two different characters, number of toes and pattern) are also sympatric in southeastern Louisiana (at least), and, in some cases, in the same pond, we are probably dealing with two separate species. It is difficult to conceive of two characters as different as number of toes and pattern that would result from a single genic element. If we were dealing with true subspecies and an area of intergradation, we should expect to find an almost complete mixture of characters in the intergrading area; that is, two-toed individuals with all variations in pattern and three-toed individuals with the same diversity. Intergradation is indicated in only three specimens out of 131 from the critical area (Series 4 and 5).

On the basis of this investigation the writer considers the three individuals to be hybrids. If there were a high proportion of mixed individuals in the critical area, the case for intergradation and a subspecific relationship would be greatly strengthened, particularly if the characters typifying the two groups were well jumbled in the intermediates. More individuals need to be collected from the critical area (Series 4 and 5).

Body proportions—The indication of species separation is further

reinforced by the fact that three-toed individuals, as a group, differ from the two-toed in proportionate limb length, and that the difference exceeds the within-group divergence (Tables 3 and 4). This is probably one of the most valuable parts of the investigation. It is not as important to note that head and tail portionalities are similar as to note that the latter reinforce and substantiate the fundamental toe and color pattern differences.

Summary

Amphiuma has been separated into two species, *Amphiuma means* and *Amphiuma tridactylum,* by Baker on the basis of number of toes, color and the ratios of total length to body length and body length to limb lengths. In deriving the mean ratios, Baker did not recognize the influence of ontogenetic variation, allometric growth or sexual dimorphism.

This writer's research was concerned with the evaluation of the three assumptions above and an attempt to determine the real differences between the two-toed groups and the three-toed groups.

Heretofore, *Amphiuma* has been separated into a maximum of three groups to be compared with each other. Goin divided *Amphiuma* into a three-toed group, a two-toed group and an intergrade group and compared these groups. The author divided 537 *Amphiuma* into six series on the basis of geographical distribution. Series 1 is composed of individuals collected west of the Mississippi River; Series 2 consists of individuals from Audubon Park; Series 3 includes those individuals within the alluvial valley east of the Mississippi River; Series 4 (three-toed) and Series 5 (two-toed) includes those *Amphiuma* within the region where the alluvial valley of the Mississippi River meets the East Gulf Coastal Plain; Series 6 is composed of individuals from the Atlantic coastal states.

The reason for this division of *Amphiuma* into series was to determine the range of variation within the three-toed series and within the two-toed series so that the differences between two-toed and three-toed *Amphiuma* could be interpreted more accurately. The Audubon Park series was separated from all other series because it is geographically isolated and the individuals of this series do not reach a size as large as those from the surrounding areas. Since it was not known whether or not the three-toed *Amphiuma* within the eastern alluvial valley of the Mississippi River were genetically similar to those west of the Mississippi, these two series were separated. Series 5 was separated from Series 6 in order to determine if there was any difference between these two two-toed groups and to determine if there was a closer relationship between Series 5 and Series 6 than between Series 4 and Series 5.

The characters used in determining the differences and/or similarities between the series are number of toes, coloration, body proportions of head length to body length, fore-limb length to body length, hind-limb length to body length and tail length to body length. The two-toed and the three-toed *Amphiuma* cannot be completely

separated into two groups by any one of the above characters. The best separation is obtained on the basis of number of toes and throat coloration.

Intergradation is indicated in only three specimens out of 131 from the critical area (Series 4 and 5). On the basis of this investigation the three individuals are considered hybrids. The indication of species separation is further reinforced by the fact that three-toed individuals, as a group, differ from the two-toed in proportionate limb length, and that the difference exceeds the within-group divergence.

Acknowledgements

I am indebted to Dr. Fred R. Cagle of the Department of Zoology, Tulane University, for suggesting this problem and for many helpful suggestions during the course of this study and the preparation of this manuscript. I am indebted to Drs. E. S. Hathaway and G. H. Penn for their critical analysis. For the loan of specimens, I wish to thank Mr. Charles M. Bogert of the American Museum of Natural History, Dr. Doris M. Cochran of the United States National Museum, Mr. Richard Etheridge of Houston, Texas, and Dr. Arnold B. Grobman of the University of Florida. Mr. Allan H. Chaney and other graduate students at Tulane University were helpful in collecting additional field samples after this research was begun.

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