

1980


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Recommended Citation

Sewell, Stephen A.; Beadles, John K.; and McDaniel, V. Rick (1980) "Systematic Status of the Fishes of Genus *Campostoma* (Cyprinidae) Inhabiting the Major Drainages of Northern Arkansas," *Journal of the Arkansas Academy of Science*: Vol. 34 , Article 28. Available at: <http://scholarworks.uark.edu/jaas/vol34/iss1/28>

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THE SYSTEMATIC STATUS OF THE FISHES OF GENUS *CAMPOSTOMA* (CYPRINIDAE) INHABITING THE MAJOR DRAINAGES OF NORTHERN ARKANSAS

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ABSTRACT

A total of 6980 morphometric and meristic data points were analyzed from specimens previously identified as *Campostoma anomalum pullum* and *Campostoma oligolepis* from all areas of their Ozark ranges in Arkansas.

Data were analyzed using the Statistical Analysis System (SAS) and IBM 370 Computer System (HASP II). An attempt was made to establish separation points through multivariate analysis (MANOVA) by taxon and watershed. Additional analyses included factor analysis and discriminant function analysis. Scale count frequencies were analyzed using computer program MINITAB.

MANOVA revealed a lack of consistent separation points, and Ozark populations were shown to be distinct and highly variable from one watershed to another. Factor analysis revealed high correlation among morphometric characters ($r > .8$), high correlation among meristic characters ($r > .8$), and biologically insignificant correlation between the two sets of data. Discriminant function analysis classified 77.57% of all Ozark specimens as *C. a. pullum*, regardless of scale count. Frequency distribution analysis of scale counts for Ozark specimens revealed a normal distribution indicative of a single-species sample. Validity of sub-specific status for taxon *oligolepis* in Arkansas Ozark populations is implied.

INTRODUCTION

Controversy over the systematic status of the central stoneroller, *Campostoma anomalum pullum* (Agassiz), and largescale stoneroller, *Campostoma anomalum oligolepis*, Hubbs and Greene, has continued since the latter was first described as *oligolepis* by Hubbs and Greene in 1935. At that time, the taxon *oligolepis* was thought to be endemic to the driftless area of Wisconsin but has since been reported in the Ozark Uplands of Arkansas and Missouri (Pflieger, 1975).

C. a. pullum is a wide-ranging form, occurring west of the Mississippi River as far as eastern Nebraska, Kansas, Oklahoma, and as far south as northcentral Mexico. This subspecies also occurs in extreme northwestern Ohio, northern Indiana, extreme southwestern Kentucky, and southwestern Mississippi (Trautman, 1957). *C. a. pullum* is found in most major drainages in Arkansas (Buchanan, 1973).

Hubbs and Greene (1935) considered taxa *pullum* and *oligolepis* to be subspecies of *C. anomalum* although little evidence of intergradation was indicated where the two occurred together in Wisconsin. Their decision was based on the intermediacy of certain characters of the Ohio stoneroller, *C. a. anomalum*, between taxa *oligolepis* and *pullum* (Pflieger, 1971), particularly scutellation.

Pflieger (1971), in examining Missouri forms of *C. a. pullum* and *C. oligolepis*, pointed out that scale counts from northern and southeastern Ozarks (the Osage, Meramac, and Gasconade rivers, and headwater diversion systems) are similar to those of Wisconsin populations reported by Hubbs and Greene (1935), but are consistently lower than those of the southern Ozarks (the White, Black, and St. Francis rivers). He further noted that variation was not clinal. Scale counts for headwater diversion systems were as low as those from the northern Ozarks, despite the proximity of populations with higher counts. Taxon *pullum* was further found to occupy several of these small Ozark headwater streams to the complete exclusion of *oligolepis*, although no locations were found where the reverse was true.

Commonly used key characters (counts of lateral line scales, circumferential scales, lateral line-circumferential sum, and predorsal scales) which have proven effective in separating the two in northern portions of the range of *oligolepis* (Burr, 1974; Burr and Smith, 1976; Smith, 1978) are inconclusive in Arkansas due to overlapping counts.

The purpose of this study, therefore, was to examine the sources and degree of morphometric and meristic variation existing between populations of the genus *Campostoma* inhabiting the waters of northern Arkansas, in an effort to gain a better understanding of the systematic position warranted by them.

METHODS AND MATERIALS

Specimens employed in this study were either collected or obtained from catalogued collections in the Arkansas State University Museum (ASUMZ), and uncatalogued specimens at the University of Arkansas, Fayetteville. Catalogued collections (ASUMZ) containing specimens from the Caddo and Saline rivers of southern Arkansas, and uncatalogued specimens from the Roanoke River, Virginia, were employed for comparison. The latter have been tentatively identified as *C. a. michauxi* (Buth and Burr, 1978).

Collections were made by use of standard minnow seines (0.64cm mesh and 1.82m depth) of 3.04, 4.57, 6.10, and 7.62 meters in length, as well as an engine-powered electroshocker (O/R Engine, Inc., type 214).

Separation for analysis was by use of the key character of Buchanan (1973) based on Pflieger (1971). This was a combined circumferential and lateral line scale count of 75-87 for *oligolepis* and 89-101 for *pullum*.

The character set used was developed from characters used in past morphometric and meristic studies (Sewell, 1979), particularly those used by Ross (1952) in separating eastern forms of *C. anomalum*, and those used by Burr (1974) in separating *Campostoma* specimens of the upper Mississippi River Valley. Specimens employed were 50mm, or more, in standard length to minimize effects of allometric growth.

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Meristic characters employed were counted by the direct count method, using an American Optical dissecting microscope (30 and 90), as well as a Casper 4X and 8X hand-held lens. An American Optical 7.5v light source was used for illumination. Meristic variables were circumferential scales, lateral line scales, predorsal scales, scales above the lateral line, anal rays and pectoral rays.

Morphometric characters were measured using Helios dial calipers, to the nearest 0.1mm. Morphometric variables were head length, trunk length, standard length, total length, snout to dorsal fin distance, snout to pectoral fin distance, snout to anal fin distance, snout to lip distance in ventral view, gape width, depth, eye to eye distance, eye diameter, and least depth of caudal peduncle.

Computer analysis was accomplished using the IBM-370 operating system at the University of Arkansas, Fayetteville. Analysis of data was by use of the Statistical Analysis System (SAS), using factor analysis, multivariate analysis of variance (MANOVA), and discriminant function analysis packages (Barr et al., 1976). MANOVA analyzed each variable between data groups and computed simple statistics for each variable in test groups, taxon and watershed. Computed *F* values and probabilities were analyzed in a determination of significance between group differences for each variable. A $P > F$ value of 0.0009 or less, was used to determine conclusive significance.

Figures, summarizing encountered variation, were computer prepared. Computer system MINITAB was used for simple mathematics and graphs (Ryan et al., 1976).

RESULTS

The first factorial axis (factor 1) accounted for 60.9-67.0% of all variation observed in the data swarm. The second factorial axis (factor 2) accounted for an additional 14.5-21.8% of all variation, for a combined total of from 75.4 to 88.8%. Due to the large degree of total variation accounted for by these two axes, remaining variation was not plotted by MANOVA, and deemed biologically insignificant.

The factor 1 axis represented an interaction of the various morphometric characteristics (values 0.77-1.0), with the pectoral ray variable loosely associated with it (values 0.58-0.89). The factor 2 axis was associated with the various meristic characteristics, with all values falling within the range of 0.61 to 1.0, except the pectoral ray variable (values -0.52 to 0.87).

Analysis of *oligolepis* populations by watershed revealed several significant differences. Head length, snout to dorsal fin distance,

snout to pectoral fin distance, snout to anal fin distance, standard length, total length, trunk length, snout to lip distance, circumferential scale count, lateral line scale count, eye to eye distance, eye diameter, and least depth of caudal peduncle were significantly different from one watershed to another. In addition, pectoral ray count, body depth, and gape width variables approached significance in their differences. MANOVA generated test criteria *F* scores of 5.39 (Hotelling-Lawley Trace), 5.02 (Pillai's Trace), and 5.21 (Wilks' Criterion). A $P > F$ of 0.0001 was observed in all three cases, and the null hypothesis of no overall watershed-related effect, rejected.

Scattergram of factor scores for this test (Fig. 1) reveals that factor 1 mean values for watersheds #2 (Black River) and #3 (White River) are highly similar, indicating a high degree of morphological similarity between these two populations, while watershed #1 (St. Francis River) is significantly displaced. Mean factor values further indicate that watershed #3 and watershed #1 specimens demonstrate the greatest degree of meristic similarity, of the three. Mean values for factor 1 in the St. Francis, Black, and White rivers were -0.7637, 0.2179, and 0.1907, respectively. Mean values for factor 2 within these systems were -0.2398, 0.3566, and -0.2226, respectively.

Analysis of taxon *pullum* populations by watershed yielded significant differences for all variables except gape width, and pectoral ray counts. MANOVA generated test criteria *F* scores of 10.51 (Hotelling-Lawley Trace), 6.84 (Pillai's Trace), and 8.58 (Wilks' Criterion). The $P > F$ in each case was 0.0001, and the null hypothesis for watershed effect, rejected.

Characteristic vectors provided by MANOVA were used to convert factor scores to adjusted canonical variate scores for the purpose of graphic representation (Fig. 2), due to the high incidence of overlap and confusion encountered. Factor 1 means provided by MANOVA, however, revealed that watershed #1 and watershed #6 (Saline River) were similar morphometrically ($\mu = -0.8568$ and -0.6590 , respectively). Similarly, watershed #2 and watershed #5 (Caddo River) grouped together ($\mu = -0.1258$ and -0.0875 , respectively), as did watersheds #3 and #4 (Roanoke River) with mean values of 0.5295 and 0.5806, respectively. Meristic values (factor 2) likewise separated three distinct groups. Watersheds #1, #2, and #3 (northern Arkansas drainages) were meristically similar ($\mu = -0.3988$, -0.6312 , and -0.2538 , respectively). The Caddo River and Roanoke River specimens were similar meristically ($\mu = -0.6025$ and 0.5031, respectively) while the Saline River specimens were significantly displaced from all others with a mean value for factor 2 of 1.3394.

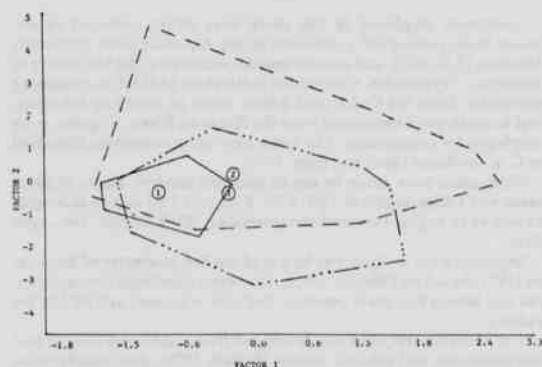


Figure 1. Scattergram of the results of factor analysis of Arkansas *Campostoma oligolepis* specimens by watershed (1 = St. Francis River, solid line; 2 = Black River, solid line; 3 = White River, dashed line; 4 = White River, dash-dot line). Numerals indicate mean position.

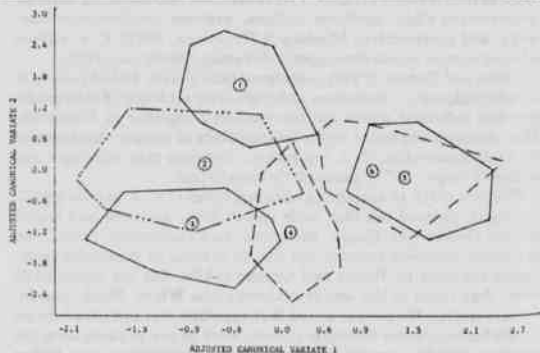


Figure 2. Scattergram of adjusted canonical variates calculated from factor analysis of selected populations of *Campostoma anomalum pullum*, by watershed (1 = St. Francis River, AR; 2 = Black River, AR; 3 = White River, AR; 4 = Roanoke River, VA; 5 = Caddo River, AR; 6 = Saline River, AR, dashed line). Mean values indicated by numeral position.

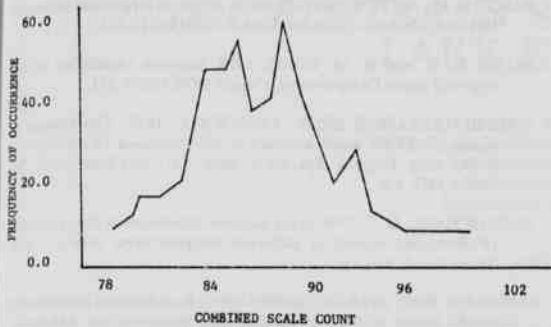


Figure 3. Frequency of occurrence of combined scale counts of lateral line and circumferential scales in genus *Campostoma* specimens from the major drainages of northern Arkansas.

Analysis by taxon within each watershed produced significant differences only in scale count variables. In the White River, the two taxa were significantly different in circumferential scale count, lateral line scale count, predorsal scale count, and scales above the lateral line. In the Black River, the two populations significantly differed only in the circumferential scale count. In the St. Francis River, significant differences were noted in circumferential scale count and lateral line scale count.

To further analyze the scale counts producing significant differences in northern Arkansas, the frequency of occurrence of each combined count of circumferential and lateral line scales within the combined ranges of the two taxa (78-100) was plotted (Fig. 3). A continuous array of values was produced from a low of 78 to a high of 100. Modal counts for the White, Black, and St. Francis rivers were 89, 86, and 84, respectively. The overall mean count for northern Arkansas specimens was 85.97. The curve produced is typical of the normal distribution of a single population (Book, 1978). In each watershed, three modes of concentration were observed (Sewell, 1979). The smaller extreme modes may represent pure line individuals, while the larger central mode may represent intergrades.

Discriminant function analysis employed reference groups from the upper Black River. All remaining specimens were analyzed as unknowns. Of those specimens originally classified as *oligolepis*, 77.6% were reclassified as *pullum*. Of those specimens originally classified as *pullum*, 23.3% were reclassified as *oligolepis*. These results indicated a mingling of overall characteristics above and below the original separation points, with the majority of all specimens examined revealing a closer relationship with *pullum* calibration group.

The correlation matrix generated with discriminant function procedure revealed all morphometric characters to be highly correlated ($r > .8$) except snout to lip distance ($r = .3$ to $.8$). All morphometric variables were poorly correlated with meristic variables (low negative values). Scales in the lateral line and circumferential scales were highly correlated ($r > .8$). Scales above the lateral line and predorsal scales were less intercorrelated ($r = .04$ to $.8$). Pectoral ray counts revealed no biologically significant correlation with any other variable ($r = -.2$ with other meristics, and $.3$ with all morphometrics).

DISCUSSION

In the original assignment of specimens into taxa for analysis, the key characters suggested by Buchanan (1973), based on Pflieger (1971), were used. These were a sum of lateral line and circumferential scales of 75 to 87 for taxon *oligolepis*, and 89 to 101 for *pullum*. However, if this separation were valid, discriminant function analysis results should have agreed with our original assignments. Further-

more, no other areas of consistent difference were detected by MANOVA.

Lateral line scale number in the genus *Campostoma* has been shown to be influenced by temperature (Carmichael and Aspinwall, 1977; Carmichael, 1979). Individuals raised at a lower mean temperature (13.9°C) were shown to have significantly more scales in the lateral line than those raised at a higher mean temperature (24.3°C). Studies in the Buffalo River (Cashner and Brown, 1977) seem to substantiate these findings, in that collections in the headwater regions with lower mean temperatures (25-28°C, to 28-31°C, downstream) contained a higher number of specimens identified as *pullum*, often to the complete exclusion of *oligolepis*. Longer developmental periods typically produce higher counts in meristic structures (Hubbs, 1926) and developmental rate has been shown by many researchers to vary directly with temperature (Barlow, 1961).

Other methods have been used in conjunction with scale counts to separate these two taxa. Coloration, tubercle pattern, and general body form of males in breeding condition were employed by Burr (1974), Smith (1978), and Pflieger (1975). In Arkansas, however, the combined lateral line and circumferential scale count used as a key character, regularly places specimens exhibiting both tubercle patterns in the same taxon (Sewell, 1979). Wiley and Collette (1970) believed nuptial tubercle patterns to be related to breeding habits. Burr and Smith (1976) suspected tubercle differences to be related to spawning behavior. Branson (1962) pointed out that extensive tubercle development in Ozark minnows (Cyprinidae) was associated either with very active spawners, or those spawning in rapidly flowing waters. Koehn (1965) found that tubercle patterns in the breeding males of the Red Shiner, *Notropis lutrensis*, were highly variable. In the absence of additional supporting characters, nuptial tubercle patterns are of little value in separating species. The breakdown of this character as a specific indicator in Arkansas *Campostoma* was further illustrated by discriminant function analysis. Coloration differences noted by Burr and Smith (1976) agree with conditions encountered in this study in that older, larger breeding males which lacked a crescent-shaped row of tubercles beside the nostrils, frequently displayed limited black pigmentation of the anal fin.

Cloutman (1976) differentiated the two taxa in the White River on the basis of parasite fauna, particularly the number of *Crassiphiala bulboglossa* Van Haitsma. Numbers of this parasite increase with the length and age of the fish, presumably as a function of exposure time. Of those specimens identified as *oligolepis* by Cloutman, 43% were over one year of age, or age group 1, while only 5% of those specimens identified as *pullum* were age group 1. All others were age group 0. A higher incidence would therefore be expected in the *oligolepis* group due to exposure time, and not necessarily due to host susceptibility.

Buth and Burr (1978) presented electrophoretic evidence in support of specific status for *oligolepis*. However, it should be noted that *oligolepis* specimens from Illinois exhibited "an unusually high degree of genetic variability, high heterozygosity levels, and shared several variate alleles with *C. a. pullum*" specimens from Illinois. *C. oligolepis* specimens from Missouri did not exhibit these genetic similarities with *pullum* specimens from Illinois; however, *pullum* specimens from Missouri were not used for comparison. Furthermore, Illinois and Missouri populations of *oligolepis* exhibited the lowest genetic similarity coefficient ($I = 0.94$) encountered in any intra-taxon test group employed. Incorporation of Missouri *pullum* specimens may have revealed the close genetic relationship observed in Illinois specimens.

During this study, no evidence was found that would indicate granting specific status to the taxon *oligolepis* in Arkansas. This conclusion was supported by the following observations: absence of consistent significant morphological differences; absence of meristic differences except those supplied with the original identification of specimens; invalidity of nuptial tubercle patterns as discriminators; presence of intergrades between previously nonintegrating taxonomic entities; and graphic curve of combined lateral line and circumferential scales atypical of separate species.

External characters which have proven effective discriminators in other parts of the range of *oligolepis* were not useful for separation of these two taxa in Arkansas. In the upper Mississippi River Valley, Burr and Smith (1976) reported 99.8% separation using the sum of circumferential and lateral line scales as a specific indicator, and a count of 85 as "break." Ecological differences reported by Burr and Smith (1976) parallel those encountered in this study, but no confident point for separation was found.

Bailey (1956) asserted that *oligolepis* is either a full species or an environmental variant, and favored the latter view. MANOVA results support this view, as indicated. Variation within the genus *Campostoma* in Arkansas appears to be regulated by highly variable environmental conditions, presumably during development, coupled with the fact that members of the genus in Arkansas are highly responsive to local environmental conditions as indicated by inter-watershed analysis. This degree of variability was reported by Burr and Smith (1976). The evident presence of intergrades in Arkansas waters further supports the view of *oligolepis* as an environmental variant. Based on our results, the validity of the trinomial *C. a. oligolepis*, originally applied by Hubbs and Greene (1935), is indicated.

ACKNOWLEDGEMENTS

Special thanks to Dr. R. P. Smith, Department of Mathematics and Physics, Arkansas State University, for his guidance and expertise. Thanks to Allen Carter and Sam Henry of the Arkansas Game and Fish Commission, and Chris T. McAllister, Department of Biological Sciences, Arkansas State University, for their assistance in the collection of specimens. Thanks also to Dr. William Matthews, University of Oklahoma Biological Station, for loan of the Roanoke River specimens.

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