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A CHARACTER ANALYSIS OF *Gobionellus boleosoma* and *G. shufeldti* (Pisces: Gobiidae) FROM THE NORTH-CENTRAL GULF OF MEXICO

Numerous ichthyologists have found *Gobionellus boleosoma*, the darter goby, and *G. shufeldti*, the freshwater goby, to be broadly complimentary in habitat affiliation where their ranges overlapped (Bailey *et al.*, 1954; Dahlberg, 1972). The freshwater goby is known from the southeast Atlantic coast from North Carolina to Florida, the northern Gulf of Mexico, Venezuela and Brazil (Gilbert and Randall, 1979). The darter goby ranges along the Atlantic coast from Chesapeake Bay to Brazil (Gilbert and Randall, 1979). Freshwater gobies typically occupy low salinity, upper estuarine marshes, while darter gobies are most plentiful in the more saline marshes and grassflats of the lower estuary and barrier islands (Dawson, 1969). Both species are common inhabitants of the muddy bottomed estuaries of the north-central Gulf of Mexico.

Traditionally, a combination of several meristic and pigmentation characters have been used to distinguish the two species (Ginsburg, 1932). Darter gobies usually possess 11 second dorsal rays and 12 anal rays. Freshwater gobies are characterized by 12 second dorsal rays and 13 anal rays. Darter gobies are further distinguished by the presence of a dark triangular patch above the pectoral fin base, and by a series of three V-shaped marks ascending from the second, third, and fourth of five elongate blotches on the side of the trunk. These characters are not expressed in *G. shufeldti*.

Variations in the diagnostic fin ray counts and pigmentation patterns have been reported by Ginsburg (1932) and Dawson (1969). Bryan *et al.* (1976), in a

study of the Atchafalaya Basin, noted the variability of these two forms within that estuary. As the diagnostic meristic characters showed some overlap and distinguishing pigmentation patterns often appeared intermediate, there was some confusion of the specific identity of specimens. When this phenotypic variability was coupled with the reported complementarity of the ranges of *G. boleosoma* and *G. shufeldti* within coastal estuaries the question arose as to whether the forms were valid species or merely ecophenotypes. A similar situation was observed by these same investigators for *Menidia* spp. within the Atchafalaya Basin, and these data were used later to synonymize *M. audens* with *M. beryllina* (Chernoff *et al.*, 1981). An analysis of the nominal species, *G. boleosoma* and *G. shufeldti*, along the north-central Gulf coast was undertaken to determine their taxonomic status.

METHODS AND MATERIALS

A total of 231 *G. boleosoma* and *G. shufeldti* from estuaries along the north-central Gulf coast were examined for six morphometric, five meristic and five qualitative characters.

The morphometric characters were: standard length, interorbital width, eye depth, eye length, head depth and head length. Measurements followed Hubbs and Lagler (1964), except for eye and head depth. The former was a measure of the expanse of the eye perpendicular to the body axis. Fleshy supraorbital crests were not included in eye measurements, but were included in measurements of interorbital width. Head depth was measured from a point bisecting the line of the interorbital width measurement, diagonally across the cheek to the lower angle of the preopercle. This variation in measurement was done to compensate

72 Short papers and notes

for the frequent outward expansion of the gular region in preserved specimens. All measurements were made with dial calipers. Standard length, head depth and head length were read to the nearest 0.1 mm. Interorbital width, eye depth and eye length measurements were made with the aid of a binocular microscope and read to the nearest 0.01 mm. Head depth, head length, eye depth, eye length and interorbital width were recorded in thousandths of the standard length for each specimen.

Meristic characters counted were: dorsal spines, dorsal rays, anal rays, left pectoral fin rays, and lateral scales. These counts followed methods outlined by Ginsburg (1932) and Dawson (1969).

The qualitative characters used were pigmentation patterns described in earlier studies (Ginsburg, 1932; Dawson,

1969) and others which we found useful in distinguishing the two forms. These characters were coded and used to construct a pattern index. Scores determined for each of a specimen's five separate pattern characters were summed to produce a single score, which was then treated as a single character in the analyses (Table 1; Fig. 1) The index was constructed so that specimens showing patterns characteristic of *G. boleosoma* received high values and those exhibiting patterns typifying *G. shufeldti* were assigned low values. Specimens with indistinct patterns due to preservation were eliminated from the analyses.

Univariate analyses were performed using the methods outlined by Hubbs and Hubbs (1953). Comparisons of means between subgroups representing pooled allopatric and pooled sympatric samples

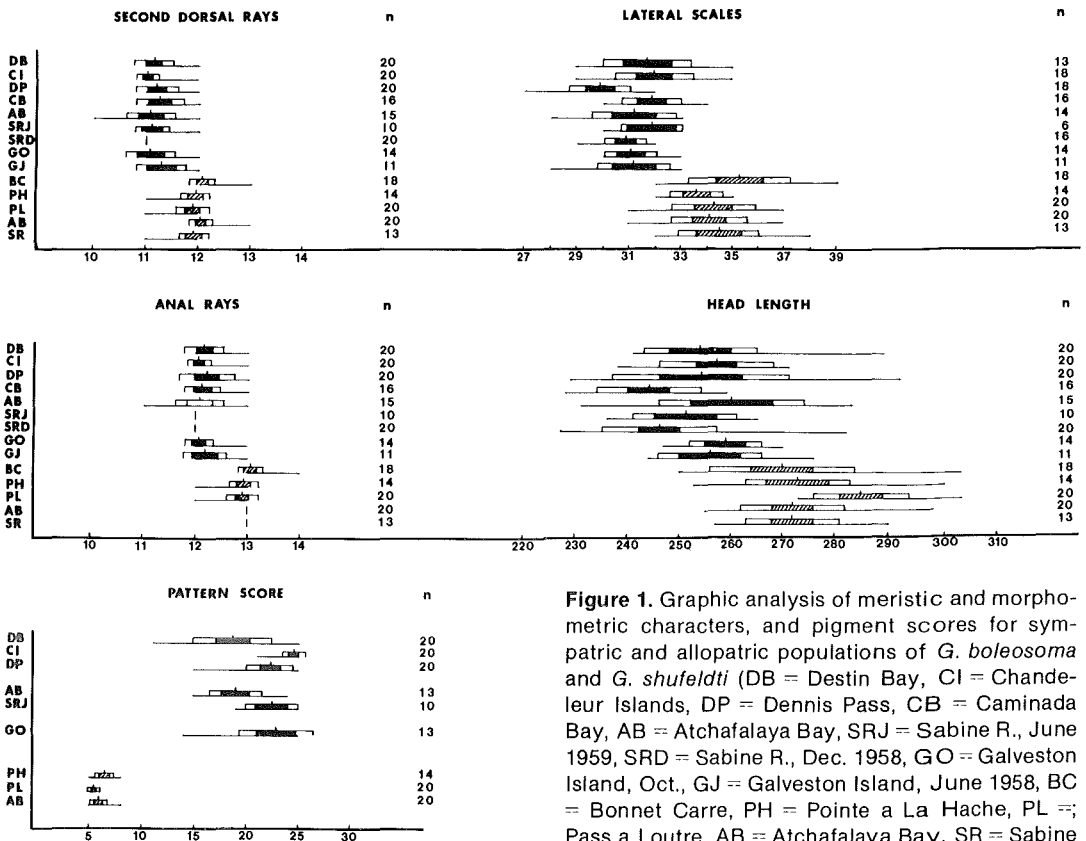


Figure 1. Graphic analysis of meristic and morphometric characters, and pigment scores for sympatric and allopatric populations of *G. boleosoma* and *G. shufeldti* (DB = Destin Bay, CI = Chandeleur Islands, DP = Dennis Pass, CB = Caminada Bay, AB = Atchafalaya Bay, SRJ = Sabine R., June 1959, SRD = Sabine R., Dec. 1958, GO = Galveston Island, Oct., GJ = Galveston Island, June 1958, BC = Bonnet Carre, PH = Pointe a La Hache, PL = Pass a Loure, AB = Atchafalaya Bay, SR = Sabine River, Dec. 1958). Solid shading of 4 SE = *G. boleosoma*; stippled shading of 4 SE = *G. shufeldti*.

Table 1. Qualitative index for pigmentation characters.

Character	Scoring	Significance
Shoulder patch	1	Absent
	3	Present, poorly defined
	5	Present, well-defined
V pattern	1	Absent
	2	Poorly pigmented, a few disconnected bars
	3	Only 1/2 patterned, 1 V plus some disconnected bars, not strongly pigmented
	4	Well-pigmented, but lacking 1 or 2 bars
	5	Fully patterned, 3 V's, strongly pigmented
Cheek bar	1	Horizontal, nearly parallel to maxillary when jaws closed
	5	Acute angle to maxillary when jaws closed, usually near 45°
Snout streaks	1	Posteriormost streak between eye and maxillary equally as dark as parallel streak immediately anterior
	5	Posteriormost streak darker, more heavily pigmented than anterior streaks; may also be wider and extend onto lower lip
Throat pigmentation	1	Absent or only on underside of lower lip
	2	Lip plus 25% of throat with melanophores
	3	Lip plus 50% of throat with melanophores
	4	Lip plus 75% of throat with melanophores
	5	Lip and throat completely covered with melanophores

of each species were made using one-tailed T-tests for those characters found distinctive for the two forms in the univariate analyses. One-tailed tests were used to minimize the possibility of accepting a false null hypothesis (i.e., that the allopatric and sympatric subgroup means of a morph were equal) and heighten the chances of accepting true differences between the means of the subgroups (Sokal and Rohlf, 1969). This would allow the determination of any significant tendency towards character intermediacy in a morph's sympatric subgroup. The significance level accepted was $p < 0.05$.

Materials examined in this study were obtained from the University of New Orleans Vertebrate Collection (UNO) and the Tulane University Museum of Natural History (TU). These collections were: BALIZE DELTA, MISSISSIPPI RIVER, Plaquemines Par., La., *G. boleosoma*, UNO 1772, *G. shufeldti*, UNO 770; ATCHAFALAYA BAY, St. Mary Par., La., *G. boleosoma*, UNO 1771, *G. shufeldti*, UNO 1770; SABINE PASS, Jefferson Co., Tx., *G. boleosoma*, TU 22183, TU 22369, *G. shufeldti*, TU 22183; CHOCTAWHATCHEE BAY, Okaloosa Co., Fl., *G. boleo-*

soma, TU 45185; CHANDELEUR ISLANDS, St. Bernard Par., La., *G. boleosoma*, TU 75392; CAMINADA BAY, Jefferson Par., *G. boleosoma*, TU uncat. (R. K. Strawn 56-42), TU 22022; POINTE A LA HACHE, MISSISSIPPI RIVER, Plaquemines Par., La., *G. shufeldti*, TU 1217; BONNET CARRE SPILLWAY, St. Charles Par., La., *G. shufeldti*, TU 266.

RESULTS

Univariate analyses indicated significant differences between the two forms in the number of second dorsal and anal rays, pigmentation pattern scores, the number of lateral scale rows and head length (Fig. 1). Variance for anal and dorsal ray counts was not noticeably increased in sympatric samples. Since diagnostic counts for soft rays in both median fins differ by one between the two species, variation in counts for one form frequently overlapped with characteristic counts for the other form. Deviation from the modal ray number in one fin was not always coupled with variation from the mode in the specimen's other fin.

Lateral scale counts for *G. boleosoma* ranged from 27 to 35. Counts for *G. shufeldti* ranged from 31 to 39. Mean values were 31.2 for *G. boleosoma* and 34.0 for *G. shufeldti* (Fig. 1). Ratios of head length to standard length ranged from 0.227 to 0.292 for *G. boleosoma* and 0.250 to 0.303 for *G. shufeldti*. Mean values were 0.256 for *G. boleosoma* and 0.277 for *G. shufeldti*.

Pigmentation pattern scores allowed the greatest separation of the two forms (Fig. 1). Deviations of sample means from perfect scores (5 for *G. shufeldti* and 25 for *G. boleosoma*) were most notable in the Choctawhatchee and Atchafalaya bay *G. boleosoma* samples. The former is from an allopatric population and the latter is from a sympatric population. As differences in the methods and length of preservation could affect pattern expression, slight differences between groups were not deemed significant. Diagnostic pigmentation pattern expression could also be influenced by ontogenetic factors, an individual's reproductive state, behavior, and the environment. The most problematic specimens to assign to one species or the other were juveniles and non-reproductive females. The most reliable pattern character allowing separation was found to be the relative intensity of snout streak pigmentation (Fig.2). This character is usable with specimens as small as 12 mm SL. In *G. shufeldti* there are three streaks between the eye and maxillary on either side of the head, all equally pigmented and wide. There are also three streaks on each side of the head in *G. boleosoma*, but the posteriormost streak at the corner of the jaw is darker and usually wider than the others, and may extend across the upper and lower lips.

Comparisons of the means of characters deemed diagnostic by the univariate analyses revealed no intermediacy in sympatric population samples

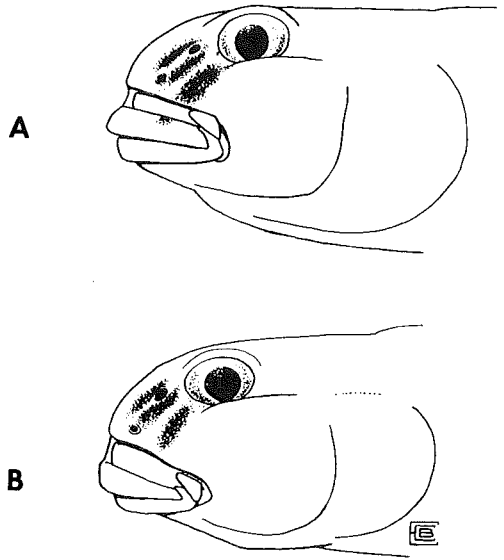


Figure 2. Snout pigmentation in (A) *G. boleosoma* and (B) *G. shufeldti*.

(Fig. 3). Arrows on the left in Fig. 3 show the direction of decreasing character value means one would expect if the subgroups represented different positions on a cline, with the sympatric subgroups similar in character state and intermediate to the two allopatric subgroups. These would be the rough expectations if the forms were actually ecophenotypes following salinity gradients within the est-

	SPECIES SUBGROUPS	EXPECTED DIRECTION OF DECREASING MEANS	MEANS	ACTUAL DIRECTION OF DECREASE	SIGNIFICANCE LEVEL
2ND DORSAL RAYS	SA	↓	12.00	↓	NS
	SS		11.98		
	BS		11.12		
	BA		11.13		
ANAL RAYS	SA	↓	13.00	↓	NS
	SS		12.96		
	BS		12.09		
	BA		12.10		
LATERAL SCALES	SA	↓	24.50	↓	NS
	SS		34.28		
	BS		30.80		
	BA		31.69		
PATTERN SCORE	SA	↑	6.43	↓	*
	SS		5.51		
	BS		20.94		
	BA		21.93		
HEAD LENGTH	SA	↓	271	↑	*
	SS		277		
	BS		253		
	BA		253		
SA-ALLOPATRIC G SHUFELDTI			BS-SYMPATRIC G BOLEOSSOMA		
SS-SYMPATRIC G SHUFELDTI			BA-ALLOPATRIC G BOLEOSSOMA		

Figure 3. One-tailed T-tests between allopatric and sympatric subgroups of *G. boleosoma* and *G. shufeldti* using characters determined distinctive in univariate analyses. See text for explanation of arrows.

uaries. Sites from which sympatric population samples were taken can be assumed to be brackish and intermediate to the freshwater in which allopatric *G. shufeldti* were collected, and the coastal marine, or nearly marine, waters from which allopatric *G. boleosoma* samples were taken. Similar expectations would be held if the two forms represented populations differentiated to the subspecific level, and showed a high degree of hybridization in sympatry. Sympatric samples of *G. boleosoma* did show a significant decrease in lateral scale counts (Fig. 1). Sympatric *G. shufeldti* samples evinced a significant decrease in pattern scores and a significant increase in head length. All of these differences were contrary to the expectations generated by the one species/ecophenotype or hybridizing subspecies models.

Differences were also noted between the degree of urogenital papilla development in males of the two species at comparable size classes (Fig. 4). The papilla was found to be relatively longer and thinner in *G. boleosoma*, and to be more

fully developed in that species at a smaller size.

DISCUSSION

Water temperatures, salinity, silt load and dissolved oxygen levels have been shown to be important factors in the phenotypic modification of fishes in natural populations (Hubbs, 1922 and 1926; Hubbs and Whitlock, 1928; Barlow, 1961). Of these factors, only gross differences in salinity distinguish habitats favored by *G. shufeldti* from those in which *G. boleosoma* predominates. The subgroup T-tests, which were designed to test correlations between character expression and salinity, indicated that there was no character intergradation in intermediate populations sampled.

If *G. boleosoma* and *G. shufeldti* were ecophenotypes, a different pattern of morphological differences from those shown would be expected. The *G. boleosoma* form (characteristic of higher salinities) would be expected to have a slower growth rate, and as a result, to attain a larger size and a greater number of median fin rays (Hubbs, 1926; Barlow, 1961). The two forms would also be expected to differ morphometrically, with the *G. boleosoma* morph having a proportionately smaller head and smaller eyes. The *G. boleosoma* form would also be expected to show more squamation. Instead, *G. shufeldti* has more median fin rays, attains a larger size, has a smaller eye and shows a greater number of lateral scale rows and greater squamation of the nape (Dawson, 1969; Fig. 1).

The only character examined in this study that would fit the typical pattern of physiological response to differing salinities within a species was the greater head length of *G. shufeldti*. Thus, there is an overall lack of fit of the recognized character states for these two forms to classical salinity modulated phenotypic

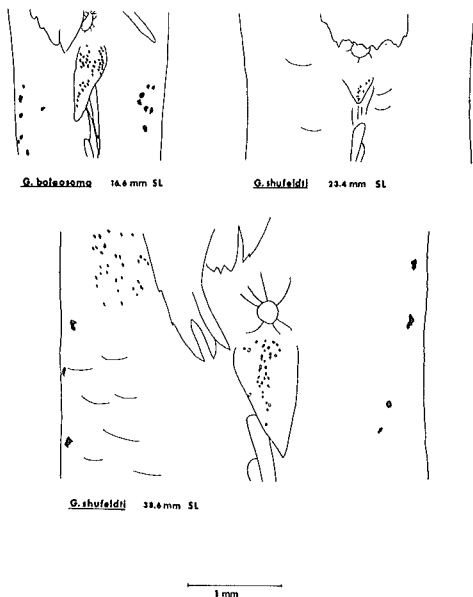


Figure 4. Comparisons of genital papillae in *G. boleosoma* and *G. shufeldti*.

variation and no evidence of inter-mediacy of these two forms in sympatric populations. Continued recognition of these two forms as valid species is supported.

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