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Source: *Copeia*, Vol. 1995, No. 3 (Aug. 18, 1995), pp. 636-649

Published by: American Society of Ichthyologists and Herpetologists

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Patterns of Morphological Variation among Endangered Populations of *Gila robusta* and *Gila cypha* (Teleostei: Cyprinidae) in the Upper Colorado River Basin

DOUGLAS M. McELROY AND MICHAEL E. DOUGLAS

The native fish fauna of the American southwest is in decline as a result of habitat destruction, disruption of natural water flows, and introduction of non-native species. The status of several members of the cyprinid genus *Gila* occurring in the upper Colorado River basin is particularly tenuous, in part because of uncertainty regarding their taxonomic status. To examine this uncertainty, we have sampled 363 specimens of *G. robusta* and *G. cypha* from eight localities in the upper Colorado River basin and the Grand Canyon and used canonical discriminant and cluster analysis to categorize patterns of morphological variation at three levels of biological organization. At the population level, all sampled populations of both species differed significantly, although there was no relationship between morphological similarity and geographic proximity of populations in either species. At the species level, the two forms were clearly distinct in morphology whether in sympatry or in allopatry. At the generic level, we found two somewhat contradictory results: (1) conspecifics from allopatric localities generally clustered together to the exclusion of heterospecifics; and (2) heterospecific populations at Cataract and Desolation canyons were more similar to one another than to allopatric conspecifics. This locality effect influencing the morphological similarities between species at these sites may be a consequence of either introgressive hybridization and/or convergent local adaptation. In general, allopatric populations of both *G. robusta* and *G. cypha* appear to represent independent evolutionary and conservation units. Populations of *Gila* should not be considered in isolation of congeners or of the environment in which they occur, and biological foresight and an emphasis on habitat conservation should be used in managing these species.

MEMBERS of the western North American cyprinid genus *Gila* represent both a taxonomic conundrum and a morphological curiosity. Extensive (but poorly defined) morphometric variation against a background of morphological specialization is characteristic of the group (Minckley, 1973; Douglas et al., 1989; Douglas, 1993), a dichotomy that has hampered attempts to elucidate species interrelationships (Douglas et al., 1989). The *G. robusta* species-complex has proven especially problematic. Several big-river species of the Colorado River basin (e.g., *G. cypha*, *G. elegans*) display extreme morphologies, presumably reflecting adaptations to life in high current regimes (Miller, 1946; Minckley, 1973; but see Kaeding et al., 1990). However, morphological variation within and among populations of these and other members of the *G. robusta* complex is extensive, and few if any morphometric characters reliably separate species (see Suttkus and Clemmer, 1977; Smith et al., 1979). Evidence (DeMarais et al., 1992; Dowling and DeMarais, 1993) that introgressive hybridization has contributed to the evolutionary history of the group at several

levels further clouds the distinction among species, and with other factors confound the identification of evolutionary units and limit confidence in sorting individual specimens to species.

Questions of species identity in *Gila* have consequences beyond evolutionary biology and taxonomy. To develop appropriate conservation strategies for these endangered or threatened taxa, it is desirable to clearly establish species identity and distinctiveness (Valdez and Clemmer, 1982; Douglas et al., 1989). However, the need to protect these fishes precludes extensive sampling and/or handling of specimens. Consequently, detailed quantitative studies on *Gila* have been limited in both number and scale, and recovery efforts remain stalled largely because of the taxonomic confusion surrounding the *G. robusta* complex (Douglas et al., 1989).

Vanicek and Kramer (1969) found differences in growth and length-weight relationships between *G. robusta* and *G. elegans* and suggested that they constituted distinct species (as opposed to subspecies of *G. robusta*). Holden and Stalnaker (1970) concluded that *G. robusta* and *G. elegans* were each morphologically homoge-

neous and represented distinct evolutionary lineages, although they considered the status of *G. cypha* somewhat ambiguous. Using a combination of morphometric and meristic characters, Smith et al. (1979) were able to differentiate museum specimens of *G. robusta*, *G. cypha*, and *G. elegans* in multivariate space. Three studies have been carried out using nondestructive sampling methods. Douglas et al. (1989) demonstrated the utility of a qualitative approach to classify individual *G. robusta* and *G. cypha* collected from the Yampa River, as well as the difficulty in separating these specimens on the basis of eight quantitative characters scored in the field. Using a different set of morphometric characters, Kaeding et al. (1990) found good concordance between taxonomic assignments made in the field and results of quantitative analyses of *G. robusta* and *G. cypha* from the upper Colorado River at Black Rocks. Douglas (1993) applied video image acquisition techniques to test sexual dimorphism in *G. cypha* and rejected the hypothesis (Holden, 1991) that the pronounced nuchal hump in this species represents a male secondary sexual characteristic.

Here we extend the use of video imaging to a consideration of morphological variation within and among populations of *G. robusta robusta* (hereafter designated *G. robusta*) and *G. cypha* in the upper Colorado River basin and the Grand Canyon. Using canonical discriminant and cluster analyses, we examine patterns of variation at three levels of biological organization. Specifically, we ask three questions. First, is there evidence of significant divergence among populations of *G. robusta* or *G. cypha*, and is there a geographic component to any patterns that may exist? Second, do specimens identified as *G. robusta* and *G. cypha* represent distinct morphologies, or does the pattern of variation represent a continuum across putative species boundaries? Third, what are the phenetic relationships among geographic populations of *G. robusta* and *G. cypha*; that is, do conspecific populations cluster together, or is there evidence of a locality effect on the similarity of groups? We discuss our findings in light of both conservation and evolutionary implications and identify avenues for future research.

MATERIALS AND METHODS

Field sampling.—Three hundred sixty-three adult *G. robusta* (n = 215) and *G. cypha* (n = 148) were collected with hoop and trammel nets and/or electroshocking from eight localities in the upper Colorado River basin and the Grand Canyon between May 1991 and Oct. 1992 (Fig. 1;

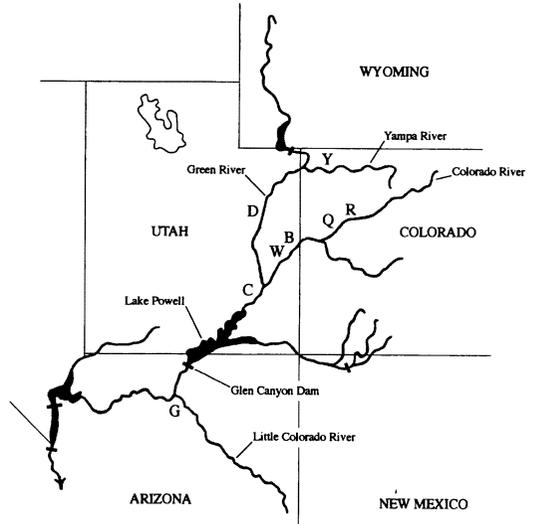


Fig. 1. Collection localities for samples of *Gila robusta* and *G. cypha* taken from the upper Colorado River basin. Population labels are identified in Table 1.

Table 1). Specimens ranged in size from 187–433 mm TL. Fish were sexed according to development of the urogenital papillus (Suttkus and Clemmer, 1977) and assigned to species by MED based on overall appearance, body proportions, fin-ray counts, and squamation (Minckley, 1973; Douglas, 1993; Douglas et al., 1989).

Sagittal views of each individual were collected on videotape following the procedures of Douglas (1993). Briefly, specimens were anaesthetized in MS-222 and placed against a neutral background with a 10-cm rule to provide scale. Background material contained a shallow depression; positioning fish in this depression minimized error associated with two-dimensional projection of a three-dimensional object (Schaefer, 1991). Dorsal and anal fins were spread, and several critical anatomical landmarks that are difficult to locate from video images were identified with insect pins. Fish were videotaped perpendicular to the midsagittal plane for approximately 10 sec using either a Sony CCD-V701 8mm or General Electric 9-9808 SE 16mm camcorder. To balance the need for image resolution against the potential for image distortion through spherical aberration, the working distance of the camera was adjusted such that each specimen filled the center two-thirds of a frame. Following videorecording, fish were placed in 19 liters of fresh river water, allowed to fully revive, and released.

TABLE 1. NUMBER OF INDIVIDUALS OF *Gila robusta* AND *G. cypha* SAMPLED FROM EACH OF EIGHT LOCALITIES IN THE UPPER COLORADO RIVER BASIN.

Population	Label*	<i>G. robusta</i>	<i>G. cypha</i>	Total
Black Rocks	B	19	25	44
Cataract Canyon	C	6	11	17
Desolation Canyon	D	24	22	46
Debeque Canyon	Q	20	0	20
Grand Canyon	G	0	28	28
Rifle	R	25	0	25
Westwater Canyon	W	56	57	113
Yampa River	Y	65	5	70
Totals		215	148	363

* Labels refer to symbols on Figure 1.

Data collection.—For each specimen, the (X,Y) coordinates of 20 anatomical landmarks (Strauss and Bookstein, 1982; Fig. 2; Appendix) were digitized directly from frozen videotape images using a VisionPlus-AT OFG frame grabber board and Morphosys morphometric analysis software, Version 1.29 OFG (Meacham, 1993). In addition, coordinates of five of 12 “helping points” (points 4, 10, 11, 13, 14; Bookstein et al., 1985) and the ends of the scale bar were similarly recorded; positions of the remaining seven helping points (points 26–32) were computed geometrically from coordinates of digitized landmarks using Morphosys. Helping points were configured primarily to quantify shape of the nuchal hump, which is highly variable in these fishes but for which few anatomical landmarks can be identified (Douglas, 1993). A modified box truss (Bookstein et al., 1985; Douglas, 1993; Fig. 2) consisting of 56 individual distances between pairs of landmarks was constructed for each specimen using Morphosys. All measurements were expressed relative to the scale bar (i.e., in absolute mm). These data formed the basis for all statistical analyses and are available upon request from the authors.

Statistical methods.—All measurements were log-transformed and subjected to principal components analysis (PCA) of the variance-covariance (VCV) matrix using NTSYS-pc (Rohlf, 1992). In all cases, the resulting first principal axis (PC I) explained a large proportion of the total variance (> 78%), and character loadings on this vector were of the same order of magnitude and uniform in sign. Given these patterns and the broad (> 2×) range of size differences among specimens, PC I was interpreted as a general size factor (Jolicoeur and Mos-

imann, 1960; Jolicoeur, 1963; Rising and Somers, 1989). To minimize effects of general size on subsequent procedures, transformed data were projected onto the space orthogonal to the first principal axis using the algorithm of Rohlf (1992), corresponding to Burnaby's (1966) method for size correction. Although this technique generates a data set of “general-size-allometry-free shape” variables (Bookstein, 1989), it is important to remember that “shape” in this context is statistically uncorrelated with our measure of general size (PCI) but is likely correlated biologically with physical size (Sundberg, 1989; Bookstein, 1989). As such, we refer to these data as size corrected rather than size free.

Size-corrected data matrices were examined for the presence of significant among-group morphological differences through canonical variates analysis (CVA) and multiple discriminant function analysis (DFA) using Statistical Analysis Systems (SAS Institute, 1985). Significance of univariate tests was assessed based on Bonferroni-adjusted probabilities. Within-group VCV matrices derived from size-corrected data were tested for homogeneity using a likelihood ratio test (Morrison, 1976). Predicted group membership was then estimated a posteriori for all specimens, based on their generalized-squared Mahalanobis distance from the centroid of each source group. Because within-group variances were homogeneous in all cases, this classification criterion was based on pooled VCV matrices. Although error rates derived from internal classification are unreliable as a measure of the efficacy of discriminant functions to assign unknown specimens, they provide a maximum bound on the classification power one might expect and allow distinctiveness of groups used in discrimination to be assessed.

Hierarchical relationships of groups in discriminant space was visualized using NTSYS-pc through cluster analysis of generalized pairwise distances among group means. Because any clustering technique produces clusters regardless of the actual structure of the data, we employed single and complete linkage clustering methods (Sneath and Sokal, 1973) as well as UPGMA. The robustness of resulting clusters was evaluated qualitatively by producing a strict consensus of all trees derived from these methods. Clusters resolved in the consensus topology are likely to be well supported (Rohlf, 1992). For intraspecific analyses (see below), the matrix correlation between canonical distances among group means and geographic proximity (in river miles) among sampling localities was examined

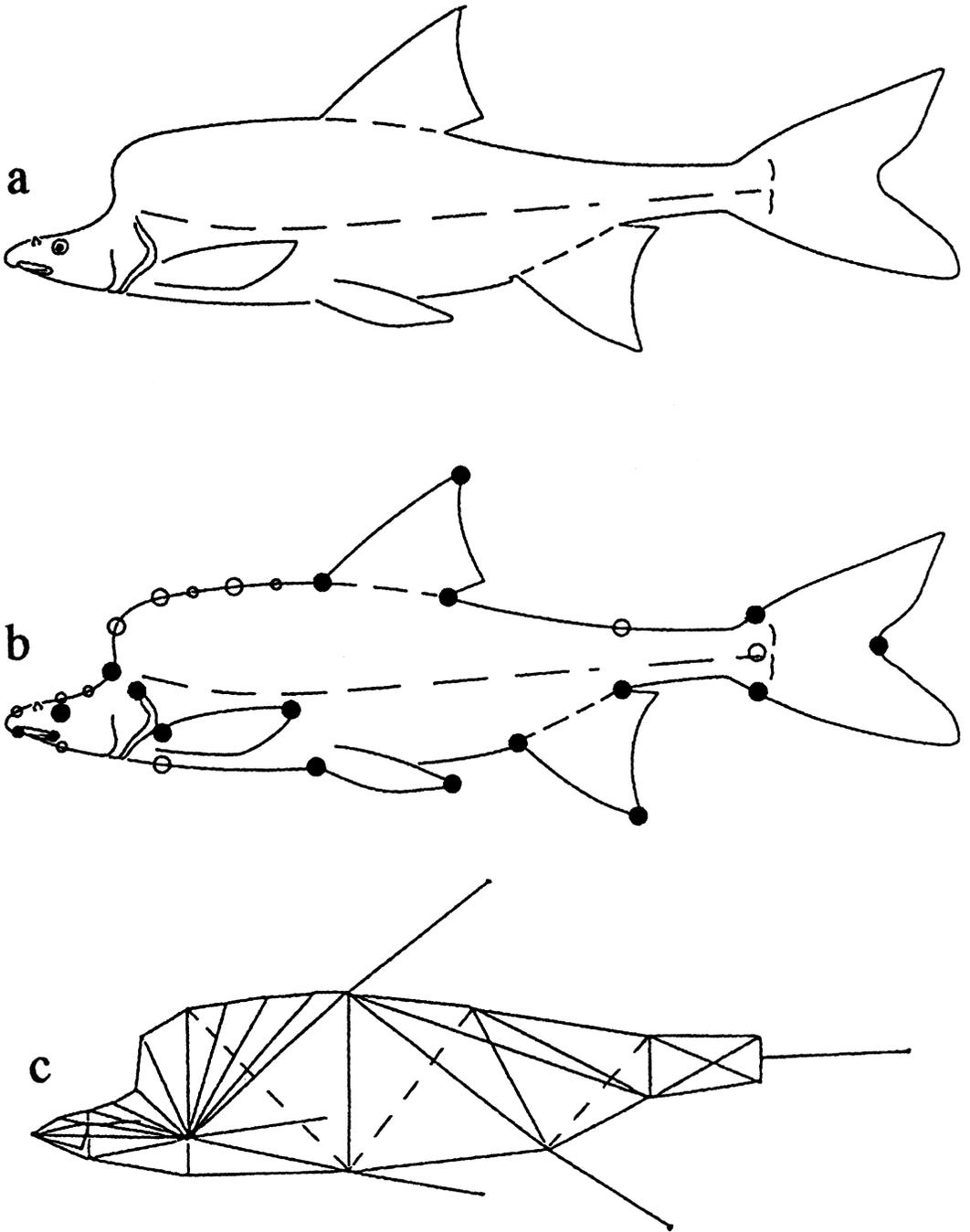


Fig. 2. Location of anatomical landmarks for *Gila robusta* and *G. cypha*: (a) generalized *G. cypha*; (b) landmark points; and (c) a 56-character truss network derived from the landmark points. In (b), closed circles identify anatomical landmarks, and open circles represent helping points designed to provide information on areas of the body for which few anatomical landmarks can be identified. The truss network in (c) includes three additional characters (dashed lines) not utilized in Douglas (1993). Definitions of landmark points are given in the Appendix.

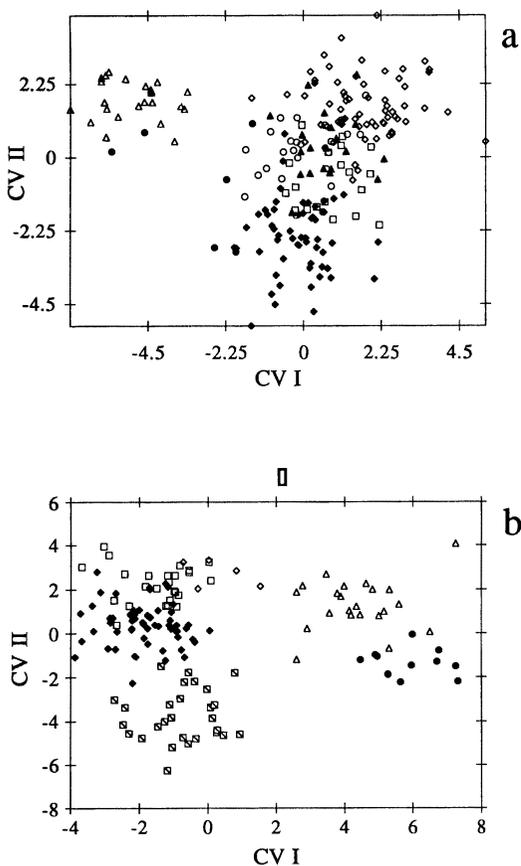


Fig. 3. Canonical variates analyses of population divergence in (a) *Gila robusta* and (b) *G. cypha*. Axes represent the first two shape factors extracted from the analyses. Significant among-group heterogeneity exists on CVI and CVII in both species, and all populations differ significantly in CV space when higher dimensions are considered. Group labels are as follows: Black Rocks (open squares), Cataract Canyon (closed circles), Desolation Canyon (open triangles), Debeque Canyon (closed triangles), Grand Canyon (hatched squares), Rifle (open circles), Westwater Canyon (closed diamonds), Yampa River (open diamonds).

using a Mantel test (Mantel, 1967); here, the normalized Mantel statistic computed from the original data was compared to a sample distribution based on 1000 permutations of the geographic matrix using NTSYS-pc (Rohlf, 1992).

Statistical analyses.—Patterns of morphological variation in *Gila* were assessed by applying the above methods to four statistical models, representing three levels of biological organization. First, the degree of population divergence within *G. robusta* and *G. cypha* was examined in separate analyses by grouping fish according to

their population of origin ($n_{\text{groups}} = 7$ for *G. robusta*, $n_{\text{groups}} = 6$ for *G. cypha*). Next, morphological distinctiveness at the species level was evaluated by categorizing individuals as *G. robusta* or *G. cypha*, independent of their population of origin. Finally, geographic patterns of morphological variation at the generic level were investigated by combining the first two analyses into a single model; that is, individuals were grouped by species and population ($n_{\text{groups}} = 13$) to simultaneously evaluate the relationship among sympatric and allopatric populations.

In each model, field identifications were used to group individuals by species. Although a more robust test of morphological variation would rely on genetic data or some other independent character to determine affinity of specimens, our approach is valid as a test of the nature and consistency of qualitative field identifications and the relative distinctiveness of species/populations among localities.

RESULTS

Variation in G. robusta.—Although principal components analysis implied limited population structure in *G. robusta*, canonical variates analysis revealed significant among-group divergence in shape. Individuals differed primarily in size; the first principal component of the variance-covariance matrix (PCI) was characterized by consistently high, positive loadings across all characters and explained 82.2% of the total variation. By contrast, PCII-IV combined explained only 47.4% of the residual variation and failed to clearly separate any populations. At least 16 latent roots (excluding PC I) contained significant information (Reyment, 1992).

Despite the high degree of overall variability evident from PCA, populations could be clearly differentiated with CVA. Forty-nine of 56 size-corrected characters displayed significant univariate differences among groups based on conservative Bonferroni-corrected criteria ($P < 0.0009$); multivariate tests of among-group differentiation also were highly significant (Wilk's Lambda = 0.0032, $F_{336,922.6} = 4.4542$, $P < 0.0001$). Characters associated with position of the pectoral and pelvic fins and body depth (vPe-OPe, VPd-Dpd, OPl-ODo) displayed the largest univariate F values. Each of the six canonical roots carried significant among-group structure ($P < 0.05$). The first canonical vector (CVI) separated the Desolation Canyon and, to a lesser extent, Cataract Canyon populations from all others (Fig. 3a). Specimens of *G. robusta* could be assigned to groups with a high degree of confidence using DFA. The overall classifica-

tion error rate was 0.042, with nine of 215 individuals misclassified. No pattern was evident among the misclassifications.

Cluster analysis provided little evidence of a geographic component to hierarchical relationships of populations (Fig. 4a). Population samples from Black Rocks and Westwater Canyon were most similar in discriminant space; these localities are separated by less than 15 river miles (Fig. 1). However, the Black Rocks/Westwater Canyon group formed part of a larger cluster containing populations occurring in different river basins and separated by over 600 river miles (Yampa River/Rifle; Fig. 1). In fact, there was no correlation between geographic proximity and morphological similarity of populations (Mantel $Z = -0.066$, $t = -0.215$, one-tailed $P = 0.475$). The Cataract and Desolation canyon populations were distinct from other populations (and from each other) under all clustering methods.

Variation in G. cypha.—As in *G. robusta*, populations of *G. cypha* could be differentiated using CVA, despite the presence of significant morphological variability. PCA results for *G. cypha* were comparable to those from *G. robusta*. PCI explained 80.1% of the total variation, and loadings on this axis reflected primarily size variation among individuals. PCII-IV combined accounted for only 48.6% of the variation not attributable to size (i.e., not explained by PCI), and at least 15 latent shape factors contained significant information. There was no evidence of population structure in these lower dimensions.

CVA of individuals of *G. cypha* revealed significant shape divergence among populations: 41 of 56 univariate comparisons showed significant among-group differences ($P < 0.0009$), and multivariate tests were highly significant (Wilk's Lambda = 0.0014, $F_{280,439.1} = 4.264$, $P < 0.0001$). The largest univariate F values were in characters associated with relative position of fins and head and body depth (OA-vIA, PudPu, OA-IA). Each of the five canonical roots captured a significant component of among-group variation. The Cataract and Desolation canyon populations were separated from all others along CVI, whereas the Grand Canyon population was distinct on CVII (Fig. 3b). As in *G. robusta*, specimens could be accurately assigned to groups using DFA. Only three of 148 individuals were misclassified (error rate = 0.020).

Hierarchical relationships of populations of *G. cypha* did not reflect geographic proximity of localities (Fig. 4b). As in *G. robusta*, samples

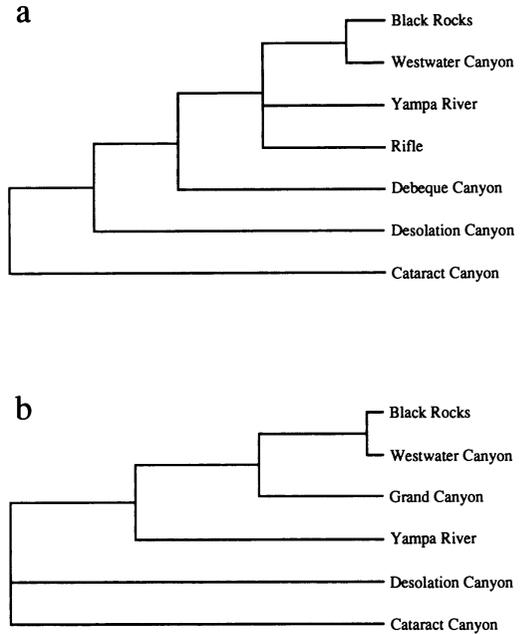


Fig. 4. Hierarchical relationships among populations of (a) *Gila robusta* and (b) *G. cypha*. Dendrograms represent the strict consensus topology derived from UPGMA, complete linkage, and single linkage clustering of generalized-squared Mahalanobis distances among group means. Branch lengths are not indicative of the true phenetic distances among nodes. In both species, there is no relationship between canonical distance and the geographic proximity of sampled populations.

of *G. cypha* from Black Rocks and Westwater Canyon were most similar, whereas both Desolation and Cataract canyon populations were distinct from all other localities. Morphological similarity was uncorrelated with geography (Mantel $Z = 0.003$, $t = 0.010$, one-tailed $P = 0.493$).

Distinctiveness of species.—Despite high variability, individuals of *G. robusta* and *G. cypha* could be discriminated readily. As in intraspecific comparisons, the first three shape factors extracted from PCA (PCII-IV) accounted for only 50.2% of the variation not attributable to size, and at least 16 latent roots explained a significant component of the total shape variation. Although some structure was evident in PCA, within-species clouds overlapped considerably (Fig. 5a).

By contrast, CVA clearly separated groups (Fig. 5b). Here, 46 of 56 univariate character comparisons were significant ($P < 0.0009$), as were multivariate tests (Wilk's Lambda = 0.207, $F_{56,306} = 20.961$, $P < 0.001$). Characters asso-

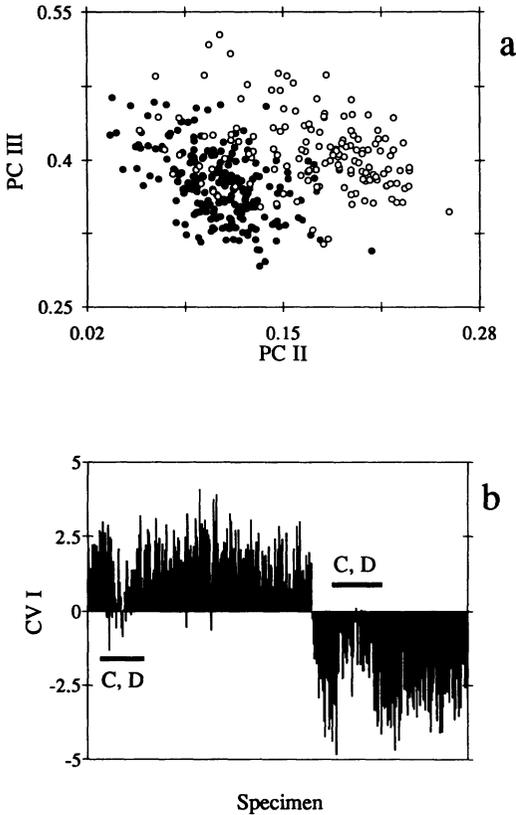


Fig. 5. Principal component (a) and canonical variates (b) analyses of morphological divergence between specimens of *Gila robusta* and *G. cypha*. In (a), axes represent the first two shape factors derived from the analysis. PCA provides evidence of structure between *G. robusta* (closed circles) and *G. cypha* (open circles), but within-species point clouds overlap considerably. In (b), the sample of *G. robusta* (specimens 1–215) clearly differs from that of *G. cypha* (specimens 216–363). Samples of both species taken from Cataract and Desolation canyons (indicated by labeled bars) are, on average, more intermediate (scores closer to zero) than are other samples.

ciated with relative size and positions of the dorsal and anal fins (ODo-OA, OA-vIA, OA-IA) were characterized by having the largest univariate F values. All specimens were correctly classified with DFA.

Geographic patterns of variation.—In addition to clear separation of species, analyses of variation at the generic level indicated the presence of a strong locality effect on relationships among several populations. The separation of groups (e.g., populations) through canonical variates analysis was good; 55 of 56 univariate character ANOVAs displayed a significant population effect ($P < 0.0009$), and multivariate tests were

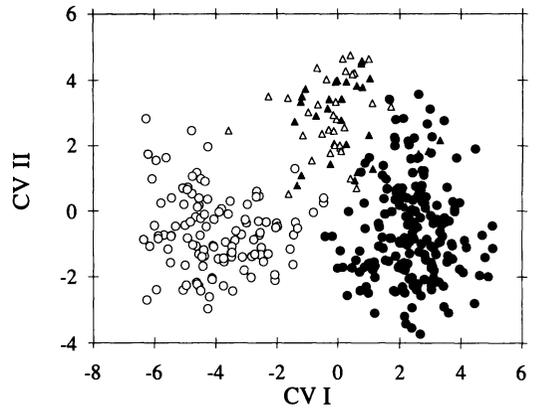


Fig. 6. Canonical variates analysis of morphological variation within and between *Gila robusta* and *G. cypha*. CV I and II represent the first two shape factors extracted from this analysis. There is a clear separation of the following: (1) a *G. robusta* group (closed circles) composed of specimens from Black Rocks, Westwater and Debeque canyons, Rifle, and the Yampa River; (2) a *G. cypha* group (open circles) representing samples from Black Rocks, Westwater and Grand canyons, and the Yampa River; and (3) a mixed group of *G. robusta* (open triangles) and *G. cypha* (closed triangles) from Cataract and Desolation canyons.

highly significant (ex. Wilk's Lambda = 0.0002, $F_{672,3510.7} = 2.314$, $P < 0.0001$). As before, characters describing fin placement (OA-vIA, ODo-OA, OA-IDo) showed the largest univariate F values. Nine of 12 canonical roots contained a significant component of among-group variation. CV I and II produced a clear separation of three groups: (1) a *G. cypha* group consisting of individuals from Black Rocks, Westwater and Grand canyons, and the Yampa River; (2) a *G. robusta* group consisting of fish from Black Rocks, Westwater and Debeque canyons, Rifle, and the Yampa River; and (3) a mixed group of *G. robusta* and *G. cypha* individuals from Desolation and Cataract canyons (Fig. 6). Greater than 92% (336 of 363) of all specimens were correctly classified using DFA. In addition, despite an overall classification error rate of 0.075 (27 of 363 misclassified), the majority (89%) of misclassifications occurred within rather than among species (Table 2). Three specimens of *G. cypha* were assigned incorrectly to *G. robusta* populations; no individuals of *G. robusta* were classified as *G. cypha*.

Cluster analysis of generalized canonical distances among populations produced two primary, and somewhat contradictory, results. First, conspecific populations tended to cluster together to the exclusion of heterospecifics (Fig. 7); samples of *G. robusta* and *G. cypha* generally

have supported the view that *G. robusta* and *G. cypha* represent distinct species, though "intergrades" have been detected in varying frequencies. Our results similarly argue for treating *G. robusta* and *G. cypha* as separate species; despite the presence of intermediates, the two forms were clearly distinct both in sympatry and in allopatry. In fact, the intermediate specimens in our sample could be assigned to species with confidence, suggesting that morphological distinctiveness of *G. robusta* and *G. cypha* is sufficient to categorize individuals despite high levels of variability. This would not necessarily be expected if the bases for a priori assignment of intermediates were more random than real (McElroy and Kornfield, 1993). Further, this variability may not be a ubiquitous feature of populations of *Gila* (Smith et al., 1979; Douglas et al., 1989; Kaeding et al., 1990): the majority of intermediates in our data set were collected from Cataract and Desolation canyons, and these populations may be subject to evolutionary forces not operating, or less important, at other localities (see below).

Only one study to date has addressed the issue of intraspecific population divergence in upper basin *Gila*. Holden and Stalnaker (1970) argued that populations of both *G. robusta* and *G. cypha* were homogeneous morphologically. However, their analysis relied on ordinal data and, thus, provided limited resolving power (Smith et al., 1979; Douglas et al., 1989). By contrast, we found subtle but significant differences in morphology among all populations in both species. This structure is overlain on patterns of variation at the species level, as a posteriori assignments of all individuals to species agreed with a priori classifications despite the existence of among-population differences. Our results also indicate that an isolation by distance model of gene flow among populations cannot account for these differences (Fig. 4): the lack of matrix correlation between canonical and river mile distances among populations of both species suggests that local processes must contribute to distinctiveness of groups. The nature of these additional biological forces (e.g., hybridization, selection, gene flow) remains to be elucidated.

The most intriguing aspect of our data concerns the relationships of populations at the generic level. In particular, although *G. robusta* and *G. cypha* are in general distinct morphologically both in sympatry and allopatry, the two species converge morphologically at Cataract and Desolation canyons. At these sites, a locality effect appears to contribute significantly to relationships among populations; by contrast, a species effect appears to dominate relationships

among other populations (Fig. 7). The Cataract and Desolation population pairs are clearly distinct from all other groups (and from one another) in all analyses. This dichotomy between species and locality effects suggests that population dynamics of *G. robusta* and *G. cypha* in Cataract and Desolation canyons may differ qualitatively from those of other populations of *Gila* in the upper basin. The structure of these populations may be driven by biological forces (see below) whose effects are less pronounced elsewhere. The uniqueness of the *G. cypha* population at Cataract Canyon has been noted previously: specimens from this area tend to be smaller, deeper bodied, and less extreme morphologically than are *G. cypha* from other localities (R. Valdez, Bio/West, Inc., pers. comm.). It has been suggested that these fish represent remnants of a much larger population that inhabited the region prior to closure of Glen Canyon Dam (Holden and Stalnaker, 1975). Time series data that might provide insight into this hypothesis are currently being analyzed to examine changes in morphology over the past century (J. Lynch, pers. comm.).

Potential bases for the locality effect.—We postulate three explanations for the morphological convergence of heterospecific populations at Desolation and Cataract canyons. First, the similarity of *G. robusta* and *G. cypha* at these localities may reflect extensive introgressive hybridization, either occurring naturally or as a result of human intervention. Second, sympatric populations may be subject to similar selection pressures within each of these localities, i.e., the observed locality effect may reflect parallel local adaptation of the two species in Desolation and Cataract canyons. Third, populations of both species at these sites may retain a high proportion of ancestral traits, and their apparent similarity may be a consequence of shared primitive characteristics. These explanations are not mutually exclusive. It is unlikely, however, that our results reflect an inability to consistently characterize species, because this would lead to poor classification of individual specimens. Although complementary genetic studies might be useful in evaluating the relative contribution of each alternative, the likelihood of each based on existing data is considered below.

Hybridization appears to have played a potentially significant role in the evolutionary dynamics of the *G. robusta* group (DeMarais et al., 1992; Dowling and DeMarais, 1993). A hybrid origin for *G. seminuda*, long suspected (Ellis, 1914; Miller, 1946; Smith et al., 1979), has been substantiated by comparison of morphological,

allozyme, and mtDNA data sets (DeMarais et al., 1992). Similarly, a discordance between well-resolved allozyme and mtDNA phylogenies, and sharing of derived molecular characters across lineages have been used to argue that "Colorado River *Gila* represent a complex of self-maintaining, genetically-distinctive species that are capable of exchanging genetic material" (Dowling and DeMarais, 1993). Our morphological data are consistent with this hypothesis. If introgressive hybridization underlies the observed locality effect, we would expect genetic studies of populations of *G. robusta* and *G. cypha* to reveal incongruous patterns of allozyme and mtDNA variation (particularly in Desolation and Cataract canyons) similar to those seen at higher taxonomic levels (Dowling and DeMarais, 1993). Research to evaluate this hypothesis is in progress (T. Dowling, pers. comm.).

Introgressive hybridization may also be a consequence of human impact. Holden and Stalnaker (1970) attributed the unique morphology of *G. cypha* in Cataract Canyon to extensive hybridization resulting from habitat changes brought about by construction of Glen Canyon Dam in 1963. Although the potential effects of such water management projects cannot and should not be discounted, it appears that hybridization among some species of *Gila* predate known human modifications of the upper Colorado basin (Dowling and DeMarais, 1993). Thus, hybridization in *Gila* is to some degree a natural phenomenon, and comparison of extant populations with museum collections made prior to known habitat alteration may help quantify the degree of human impact in Cataract Canyon and elsewhere.

Members of the genus *Gila* have long been assumed to show adaptations to local habitat conditions, both within (Smith et al., 1979) and among (Miller, 1946; Holden and Stalnaker, 1970; Rinne, 1976) species. Miller (1946) suggested that a number of putative taxa of the *G. robusta* group in fact constituted ecological subspecies. Subsequent studies failed to support this hypothesis (Holden and Stalnaker, 1970; Rinne, 1976; Smith et al., 1979). Nevertheless, the degree of spatial heterogeneity may influence both morphological and species diversity of *Gila* at a given locality (Smith et al., 1979; Grant and Grant, 1989). Desolation and Cataract canyons are both extremely energetic habitats (R. Valdez, pers. comm.), characterized by rapid and turbulent flow regimes. Such conditions may impose strong selection for a common morphology in both species, whereas less severe habitats may allow several distinct morphologies to persist. Even so, our data indicate that

fish of both species from Desolation and Cataract canyons are relatively more *robusta*-like in morphology; this would not be expected if the morphology of *G. cypha* indeed reflects adaptation to high current regimes (Miller, 1946; Minckley, 1973; Kaeding et al., 1990) and conditions at Desolation and Cataract canyons favor such adaptations. A local adaptation scenario for the locality effect observed here would be supported if genetic studies provided no evidence of introgression between *G. robusta* and *G. cypha* at the population level; however, evidence for hybridization would not in itself preclude the concomitant existence of local adaptation.

Symplesiomorphy seems an unlikely explanation for the similarity of *G. robusta* and *G. cypha* at Desolation and Cataract canyons. If these areas support relatively ancestral morphotypes, we would expect that, in addition to similarity of heterospecifics within localities, fish from the two localities would be similar to one another. This is not the case; although both Cataract and Desolation canyons contain *robusta*-like forms, the node separating conspecifics from these localities is deep. This implies that the two sites support morphologically distinct forms of both species. Nevertheless, rejecting this hypothesis with any confidence is problematic given the paucity of cladistically informative characters capable of differentiating species of *Gila* (Suttkus and Clemmer, 1977; Smith et al., 1979).

Implications for conservation.—The existence of significant population divergence in both *G. robusta* and *G. cypha* suggests that all populations represent (to some degree) independent evolutionary, and thus conservation, units. Although this is most apparent with respect to Desolation and Cataract canyon populations of both species, all populations are distinct. Whether this divergence is a consequence of local adaptation, hybridization, or genetic drift (or any combination) cannot be ascertained from our data. The lack of a consistent geographic component to relationships among populations, however, suggests that an isolation by distance scenario is insufficient to explain the results. Based on these findings, it is clear that caution and biological foresight must be employed when making management decisions.

We also emphasize that the existence of hybridization, if confirmed by genetic studies, does not imply that introgressed populations are less valuable from a conservation standpoint, for two reasons. First, it appears that hybridization in *Gila* represents to some degree an evolutionary

rather than anthropogenic phenomenon (Dowling and DeMarais, 1993). Second, the two species remain distinct despite their putative convergence; at both Desolation and Cataract canyons, *G. robusta* and *G. cypha* can be clearly discriminated. These results may suggest that selection is of sufficient magnitude to prevent genetic homogenization or swamping (Haldane, 1948; Endler, 1977; Grant and Grant, 1989). Gene flow among *Gila* species may facilitate local adaptation by providing additional variation upon which selection can act (Ehrlich and Raven, 1969; Slatkin, 1987; Dowling and DeMarais, 1993), as has been suggested previously for a range of organisms (Lewontin and Birch, 1966; Gill, 1980; Grant and Grant, 1989).

The potential for introgressive hybridization and/or local adaptation suggests that populations of *Gila* cannot be considered in isolation of congeners or of the environment in which they occur. We echo the view espoused by Grant and Grant (1989) that "maintaining communities intact could be essential for the long-term persistence of their members. It is not enough to concentrate on one or two species of interest unless, by conserving them, all others are conserved as well." Clearly, such an approach will require an increased emphasis on habitat conservation.

Future directions.—Our data point to the need for additional studies of *Gila* on at least two fronts. First, genetic studies are required to evaluate the potential importance of introgressive hybridization and local adaptation to morphological variation within and among populations. A better understanding of the relationship between genotype and phenotype in these fishes is crucial to resolving the status of intermediate specimens and to quantifying the extent of human intervention (Kaeding et al., 1990). Second, the nature and range of morphological variation among upper Colorado basin populations (particularly those of *G. robusta*) should be compared to that present in the lower basin. Only by considering additional species and populations can the taxonomic confusion surrounding upper basin *Gila* be resolved and appropriate management strategies then be implemented.

ACKNOWLEDGMENTS

The authors recognize numerous individuals from the following agencies, organizations, and/or institutions for assistance with organizing river trips, obtaining specimens, identifying and filming fishes, and recording data: Arizona

Game and Fish Department (Phoenix); Arizona State University (Tempe); Bio/West, Inc. (Logan, UT); Brigham Young University (Provo, UT); Bureau of Reclamation (Salt Lake City, UT); Colorado Department of Wildlife (Denver; Grand Junction); National Museum of Natural History (Washington, DC); U.S. Fish and Wildlife Service (Parker, AZ; Grand Junction, CO; Salt Lake City, UT); and Utah Department of Wildlife Resources (Moab; Price; Salt Lake City; Vernal). Without such assistance, this project could not have been implemented. Scientific collecting permits are also acknowledged from the states of Arizona, Colorado, Utah, and from the U.S. National Park Service, as is a USFWS Endangered Species subpermit (PRT-676811). This research was supported by Bureau of Reclamation (1-CS-40-0970A).

LITERATURE CITED

- BOOKSTEIN, F. L. 1989. "Size and shape": a comment on semantics. *Syst. Zool.* 38:173–180.
- , B. CHERNOFF, R. L. ELDER, J. M. HUMPHRIES JR., G. R. SMITH, AND R. E. STRAUSS. 1985. Morphometrics in evolutionary biology. The geometry of size and shape change, with examples from fishes. *Natl. Acad. Sci. Philadelphia Spec. Publ.* 15:1–277.
- BURNABY, T. P. 1966. Growth-invariant discriminant functions and generalized distances. *Biometrics* 22: 96–110.
- DEMARAIS, B. D., T. E. DOWLING, M. E. DOUGLAS, W. L. MINCKLEY, AND P. C. MARSH. 1992. Origin of *Gila seminuda* (Teleostei: Cyprinidae) through introgressive hybridization: implications for evolution and conservation. *Proc. Natl. Acad. Sci. USA* 89:2747–2751.
- DOUGLAS, M. E. 1993. An analysis of sexual dimorphism in an endangered cyprinid fish (*Gila cypha* Miller) using video image technology. *Copeia* 1993: 334–343.
- , W. L. MINCKLEY, AND H. M. TYUS. 1989. Qualitative characters, identification of Colorado River chubs (Cyprinidae: Genus *Gila*) and the "art of seeing well." *Ibid.* 1989:653–662.
- DOWLING, T. E., AND B. D. DEMARAIS. 1993. Evolutionary significance of introgressive hybridization in cyprinid fishes. *Nature* 362:444–446.
- EHRlich, P. R., AND P. H. RAVEN. 1969. Differentiation of populations. *Science* 165:1228–1232.
- ELLIS, M. M. 1914. *Fishes of Colorado*. Univ. Colorado Studies Ser. 11:1–136.
- ENDLER, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, NJ.
- GILL, F. B. 1980. Historical aspects of hybridization between blue-winged and golden-winged warblers. *Auk* 97:1–18.
- GRANT, B. R., AND P. R. GRANT. 1989. Evolutionary dynamics of a natural population. Univ. Chicago Press, Chicago.

- HALDANE, J. B. S. 1948. The theory of a cline. *J. Genetics* 48:277-84.
- HOLDEN, P. B. 1991. Ghosts of the Green River: impacts of Green River poisoning on management of native fishes, p. 43-54. *In: Battle against extinction: native fish management in the American west.* W. L. Minckley and J. E. Deacon (eds.). Univ. of Arizona Press, Tucson.
- , AND C. B. STALNAKER. 1970. Systematic studies of the cyprinid genus *Gila*, in the Upper Colorado River Basin. *Copeia* 1970:409-420.
- , AND ———. 1975. Distribution and abundance of mainstream fishes of the middle and upper Colorado River basins, 1967-1973. *Trans. Amer. Fish. Soc.* 104:217-231.
- JOLICOEUR, P. 1963. The multivariate generalization of the allometry equation. *Biometrics* 19:497-499.
- , AND J. E. MOSIMANN. 1960. Size and shape variation in the painted turtle, a principal component analysis. *Growth* 24:339-354.
- KAEDING, L. R., B. D. BURDICK, P. A. SCHRADER, AND C. W. McADA. 1990. Temporal and spatial relations between the spawning of humpback chub and roundtail chub in the Upper Colorado River. *Trans. Amer. Fish. Soc.* 119:135-144.
- LEWONTIN, R. C., AND L. C. BIRCH. 1966. Hybridization as a source of variation for adaptation to new environments. *Evolution* 20:315-336.
- MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27: 209-220.
- MCELROY, D. M., AND I. KORNFIELD. 1993. Novel jaw morphology in hybrids between *Pseudotropheus zebra* and *Labeotropheus fuelleborni* (Teleostei: Cichlidae) from Lake Malawi, Africa. *Copeia* 1993:933-945.
- MEACHAM, C. A. 1993. MorphSys: an interactive machine vision program for acquisition of morphometric data, p. 393-402. *In: Advances in computer methods for systematic biology.* Artificial intelligence, databases, computer vision. R. Fortuner (ed.). Johns Hopkins Univ. Press, Baltimore, MD.
- MILLER, R. R. 1946. *Gila cypha*, a remarkable new species of cyprinid fish from the Colorado River in Grand Canyon, Arizona. *J. Wash. Acad. Sci.* 36: 409-415.
- MINCKLEY, W. L. 1973. *Fishes of Arizona.* Arizona Game and Fish Dept., Phoenix.
- MORRISON, D. F. 1976. *Multivariate statistical methods.* 2d ed. McGraw-Hill, New York.
- REYMENT, R. A. 1992. *Multivariate paleobiology.* Pergamon Press, New York.
- RINNE, J. N. 1976. Cyprinid fishes of the genus *Gila* from the Lower Colorado River Basin. *Wassman. J. Biol.* 34:65-107.
- RISING, J. D., AND K. M. SOMERS. 1989. The measurement of overall body size in birds. *Auk* 106: 666-674.
- ROHLF, F. J. 1992. NTSYS-pc, Version 1.70. Exeter Software, Setauket, NY.
- SAS INSTITUTE. 1985. SAS user's guide: statistics. Version 5.0. Statistical Analysis Systems Institute, Inc., Cary, NC.
- SCHAEFER, S. A. 1991. Morphometric investigations in cyprinid biology, p. 55-82. *In: Cyprinid fishes: systematics, biology and exploitation.* I. J. Winfield and J. S. Nelson (eds.). Chapman and Hall, New York.
- SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236:787-792.
- SMITH, G. R., R. R. MILLER, AND W. D. SABLE. 1979. Species relationships among fishes of the genus *Gila* in the Upper Colorado River drainage. *U.S. Natl. Park Ser. Trans. Proc., Ser.* 5:613-623.
- SNEATH, P. H. A., AND R. R. SOKAL. 1973. *Numerical taxonomy.* W. H. Freeman, San Francisco, CA.
- STRAUSS, R. E., AND F. L. BOOKSTEIN. 1982. The truss: body form reconstruction in morphometrics. *Syst. Zool.* 31:113-135.
- SUNDBERG, P. 1989. Shape and size-constrained principal components analysis. *Ibid.* 38:166-168.
- SUTTKUS, R. D., AND G. H. CLEMMER. 1977. The humpback chub, *Gila cypha*, in the Grand Canyon area of the Colorado River. *Occ. Papers Tulane Univ. Mus. Nat. Hist.* 1:1-30.
- VALDEZ, R. A., AND G. H. CLEMMER. 1982. Life history and prospects for recovery of the humpback chub and bonytail chub, p. 109-119. *In: Fishes of the Upper Colorado River system: present and future.* W. H. Miller, H. M. Tyus, and C. A. Carlson (eds.). American Fisheries Society, Western Division, Bethesda, MD.
- VANICEK, D. C., AND R. H. KRAMER. 1969. Life history of the Colorado squawfish, *Ptychocheilus lucius*, and the Colorado chub, *Gila robusta*, in the Green river in Dinosaur National Monument, 1964-1966. *Trans. Amer. Fish. Soc.* 98:193-208.

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APPENDIX. ANATOMICAL LANDMARKS CHARACTERIZED FOR SPECIMENS OF *Gila robusta* AND *G. cypha*. (X, Y) coordinates of all anatomical landmarks (Type A) and five of the 12 helping points (Type H; 4, 10–11, 13–14) were digitized directly from frozen videotape images using Morphosys; coordinates of the remaining helping points (Type H; 26–32) were computed geometrically from positions of digitized landmarks using Morphosys. Data were used to construct a modified box truss for each specimen (Douglas, 1993). Names and acronyms for each landmark follow Douglas (1993), except where indicated.

Landmark	Acronym	Type ^a	Description
Ventral peduncle	VPd	A	Posteroventral junction of caudal peduncle with most anterior procurrent caudal-fin ray
Dorsal peduncle	DPd	A	Posteriordorsal junction of caudal peduncle with most anterior procurrent caudal-fin ray
Insertion of anal	IA	A	Posteriormost junction of base of anal fin with body at presumed midsagittal line
Vertical of IA	vIA	H	Intersection of a line drawn tangent to IA and perpendicular to long body axis with dorsal edge of body at presumed midsagittal line
Origin of anal	OA	A	Anteriormost point where anal fin contacts body at presumed midsagittal line
Insertion of dorsal	IDo	A	Posteriormost junction of base of dorsal fin with body at presumed midsagittal line
Origin of pelvic	OPl	A	Anteriormost junction of pelvic fin with body, representing presumed junction of first pelvic-fin ray with pelvic girdle
Origin of dorsal	ODo	A	Anteriormost point where dorsal fin contacts body at presumed midsagittal line
Origin of pectoral	OPe	A	Center of a circle of greatest curvature at junction of pectoral-fin rays with pectoral girdle, representing presumed junction of first pectoral-fin ray with scapula
Vertical of pectoral	vPe	H	Intersection of a line drawn tangent to OPe and perpendicular to long body axis with dorsal edge of body at presumed midsagittal line
Descent of pectoral	dPe	H	Intersection of a line drawn tangent to OPe and perpendicular to long body axis with ventral edge of body at presumed midsagittal line
Pupil	Pu	A	Center of eye
Descent of pupil	dPu	H	Intersection of a line drawn tangent to Pu and perpendicular to long body axis with ventral edge of body at presumed midsagittal line
Vertical of pupil	vPu	H	Intersection of a line drawn tangent to Pu and perpendicular to long body axis with dorsal edge of body at presumed midsagittal line
Mouth corner	MC	A	Posteriorventralmost point on upper lip, at its apparent junction with lower lip
Snout	Sn	A	Anteriormost point of upper lip at implied symphysis of left and right premaxillae
Anterior eye margin	AEye	A	Anteriormost point on orbit as aligned with long body axis
Posterior eye margin	PEye	A	Posteriormost point on orbit as aligned with long body axis
Upper operculum	UpOp	A	Anterodorsalmost tip of operculum at its junction with head
Nape	Na	A	Posterodorsal end of head, at junction of scaled and unscaled regions of skin
Tip of pectoral	TPe ^b	A	Distal tip of first pectoral-fin ray
Tip of dorsal	TDo ^b	A	Distal tip of first dorsal-fin ray
Tip of pelvic	TPl ^b	A	Distal tip of first pelvic-fin ray

APPENDIX. CONTINUED.

Landmark	Acronym	Type ^a	Description
Fork	Fk	A	Antermost point of trailing edge of caudal fin
Tip of anal ¼ (ODo-vPe)	TA ^b	A H	Distal end of first anal-fin ray Geometric point ¼ of way along dorsal contour of body from ODo to vPe at presumed midsagittal line
½ (ODo-vPe)		H	Geometric point ½ of way along dorsal contour of body from Odo to vPe at presumed midsagittal line
¾ (ODo-vPe)		H	Geometric point ¾ of way along dorsal contour of body from Odo to vPe at presumed midsagittal line
½ (vPe-Na)		H	Geometric point ½ of way along dorsal contour of body from vPe to Na at presumed midsagittal line
½ (Na-VPu)		H	Geometric point ½ of way along dorsal contour of body from Na to vPu at presumed midsagittal line
½ (vPu-Sn)		H	Geometric point ½ of way along dorsal contour of body from vPu to Sn at presumed midsagittal line
½ (VPd-Dpd)		H	Geometric point halfway along line connecting VPd and DPd

^a A = Anatomical landmark; H = Helping point.

^b These acronyms do not appear in Douglas (1993).