

## Use of Geometric Morphometrics to Differentiate *Gila* (Cyprinidae) within the Upper Colorado River Basin<sup>1</sup>

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Video images of 215 adult *Gila robusta* and 148 endangered *Gila cypha* were collected from May 1991–October 1992 at eight Colorado River basin localities (seven upper basins and one lower basin). The two species were sympatric at five of these locations; *G. robusta* was absent at one site, whereas *G. cypha* was missing at two others. Sagittal views of each individual were videotaped and 25 morphological points (15 anatomical landmarks and 10 helping points) identified. Bookstein shape coordinates were calculated from Cartesian coordinates of these landmarks and points, whereas centroid size was used as a measure of body size. Shape differences were evaluated among populations of each species using MANOVA and canonical variates analysis. In *G. cypha*, variation encompassed three aspects: nuchal hump (most pronounced in Grand Canyon forms), relative head size (larger in Cataract Canyon forms), and caudal peduncle dimensions (shorter with a tapering depth in Cataract Canyon forms but longer and uniformly deeper in those from Desolation Canyon). Nuchal development in *G. robusta* is slight, hence only head and peduncle dimensions distinguished populations. Those individuals from Cataract Canyon had relatively shorter peduncles that (again) tapered in depth from anterior to posterior, whereas *G. robusta* from Desolation Canyon possessed peduncles that were much longer and of uniform depth. Specimens from Debeque and Rifle Canyons had proportionally smaller heads. Variation among all 13 populations (i.e., both species together) was evaluated using relative warp analysis, with *G. cypha* and *G. robusta* clearly separated at all sympatric locations except those from Desolation and Cataract Canyons. Here, body shapes of the two species converged. Overall, shape variation in both species is clinal. Although results from our geometric morphometric analysis were statistically similar to those based on distances derived from a truss analysis, the geometric approach visually demonstrated phenotypic differences among populations and species and this, in turn, has management implications.

AS a discipline, multivariate morphometrics (sensu stricto) is just four decades old (Jolicoeur and Mosimann, 1960; Blackith and Reyment, 1971). Arguably, it has had three revivals during this period. The first was an improvement in shape quantification, with nonorthogonal distances being combined with horizontal/vertical ones to form a superimposed truss (i.e., a series of abutting triangles) over the shape in question (Strauss and Bookstein, 1982). The second was an analytical refinement in the quantification of body size and shape, which permitted each to be analyzed separately (Humphries et al., 1981; Rohlf and Bookstein, 1987; Bookstein, 1989a). The final was, appropriately enough, a return to an older, more intuitive but (until recently) computationally intractable methodology espoused by D'Arcy Wentworth Thompson (1917). This new approach now emphasizes three aspects: the exact definition of anatomical homologies (or land-

marks) among forms; the quantification of these landmarks in shape space; and (as in Thompson's case) the use of deformation grids among forms to actually visualize landmark-by-landmark transformations (i.e., to visualize and to quantify shape change as one form is superimposed onto another). This new synthesis, aptly termed geometric morphometrics, was formulated initially by Bookstein et al. (1985) and continued to evolve through collaboration with other researchers, particularly during a series of workshops (e.g., Rohlf and Bookstein, 1990; Marcus et al., 1993, 1996). Its uses now extend into ecological applications (Adams and Rohlf, 2000). The mathematical tools for this reedification are provided in Bookstein (1991), whereas a brief, readable synopsis is given by Rohlf and Marcus (1993). The historical development of geometric morphometrics is provided by Marcus and Corti (1996).

As a technique, geometric morphometrics is characterized by the following (as per Rohlf and Marcus, 1993): Capture of 2-dimensional (i.e., 2-D) or 3-D coordinates from previously defined

<sup>1</sup> This paper is dedicated to F. James Rohlf on the occasion of his 65th birthday.

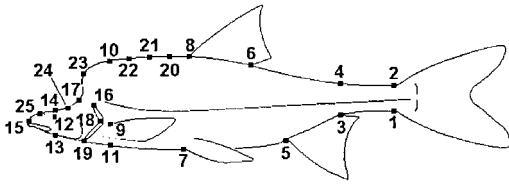


Fig. 1. Landmarks used in the current study. Definitions are given in text or in McElroy and Douglas (1995).

morphological landmarks on images of biological specimens (as in Douglas, 1993). Landmarks are (most often) homologous from image to image, thus permitting a detailed analysis of how the former shift relative to one another as shapes are contrasted. Bookstein (1991:55–87) discusses types of landmarks, their configurations, and limitations. Once coordinates are obtained, geometric relationships are clarified by fitting the former to a function (such as a thin-plate spline, see below). The parameters of a thin-plate spline transformation can themselves be used as variables in conventional multivariate analyses (as done herein). Interestingly enough, changes in form resulting from size and uniform shape alterations (i.e., affine transformation) can now be easily separated from those resulting from nonhomogeneous changes (i.e., nonaffine or local deformations). This is also demonstrated herein. Nonaffine transformations can be further split into partial warps or geometrically orthogonal components that correspond to deformations at different scales. It has been suggested that the relative contributions of these orthogonal components to overall shape change can, in turn, be employed as taxonomic characters (Zelditch et al., 1992, 1995). However, this point is disputed (Bookstein, 1994; Naylor, 1996a; Rohlf, 1998). Other methods (such as Procrustes or finite element scaling analyses; Cheverud and Richtsmeier, 1986; Rohlf and Slice, 1990) can also be used to fit differences in landmark positions among organisms.

Although there are differences among the methods noted above, all will usually lead to similar results particularly given the levels of shape variation normally found in biological data (Rohlf, 1999:212). However, to test this hypothesis, Rohlf (2000) used simulations to compare these (and other) methods in terms of type I error rates and their power for distinguishing among shape differences. He found Procrustes-based analyses superior to other procedures, and these in turn are now favored by most researchers today. However, Bookstein shape coordinates did almost as well as Pro-

TABLE 1. MATERIAL EXAMINED.

Site	Codes	<i>Gila cypha</i>	<i>Gila robusta</i>
Black Rocks Cn, UT	BR	25	19
Cataract Cn, UT	CC	11	6
Desolation Cn, UT	DC	22	24
Little CO River, AZ	LC	28	—
Westwater Cn, UT	WW	57	56
Yampa River, CO	YR	5	65
DeBeque Cn, CO	DB	—	20
Rifle Cn, CO	RI	—	25

crustes-based methods, as long as shape variation was small and the baseline reasonable. The latter aspect is judged most crucial, in that baselines defined by closely set landmarks displayed quite low power.

An important aspect of a geometric morphometric analysis is that results can be displayed visually in terms of the 2-D or 3-D space of the organism. These are easier to interpret on a comparative basis because they are configured as shapes within a coordinate system (see below). As such, they provide an analytical and graphical means to decompose phenotypic variation at different spatial scales (i.e., highly localized subregions vs globally diffuse forms; Naylor, 1996b).

In this investigation, a geometric morphological analysis is conducted on two species of western North American cyprinid fishes (*Gila cypha* and *Gila robusta*) rich in phenotypic shape (Fig. 1). The practicality of this endeavor is paramount in that the former is endangered, whereas the latter is considered a “species of concern.” Results from these analyses are then compared to a more traditional analysis of the same individuals employing distance measures extracted from a truss (McElroy and Douglas, 1995). The efficacy of the geometric approach is then evaluated relative to the truss analysis and conclusions from the two studies compared. The hypothesis under test is that significant differences exist between results of the two types of analyses.

#### MATERIALS AND METHODS

*Video images.*—The database consists of video images of adult *G. robusta* ( $n = 215$ ) and *G. cypha* ( $n = 148$ ) collected from eight localities in the upper Colorado River basin during a 17-month span (May 1991 to October 1992). The two species were sympatric at five of these locations; *G. robusta* was absent at one additional site, whereas *G. cypha* was missing at two others (Table 1). Sagittal views (left side) of each individual were

videotaped following procedures outlined in Douglas (1993) and McElroy and Douglas (1995). Specimens were allocated to species based on overall appearance coupled with morphometric and meristic characters (Douglas et al., 1989; Douglas, 1993). Allocations were done by consensus among several collaborating researchers (see Acknowledgments).

**Data collection.**—For each specimen, Cartesian coordinates of 15 landmarks (LMs) were digitized directly from a frozen video image using a VisionPlus-AT OFG frame-grabber board (Imaging Technology) and Morphosys 1.20-OFG software (Meacham, 1993). An additional four “helping points” (points 10, 11, 13, 14; Bookstein, 1991) were similarly recorded. Positions of the remaining six helping points (points 20–25) were computed geometrically from coordinates of digitized landmarks using Morphosys. Helping points were primarily derived to assist in quantifying shape of the nuchal hump (as per McElroy and Douglas, 1995). Eleven (of 15) LMs were used by McElroy and Douglas (1995) and are defined therein. The remaining four [i.e., LM4 (vertical of insertion of anal fin); LM15 (tip of snout); LM18 (posterior border of operculum); LM19 (ventral border of operculum; Fig. 1)] are unique to this study. All LMs and points are illustrated in Figure 1.

**Statistical analyses.**—In common with Bookstein (1991) and others (e.g., Loy et al., 1993; Carpenter, 1996), centroid-size is used as a measure of overall body size, whereas body shape is quantified using shape coordinates. Centroid size is defined as the square root of the summed, squared distance of all landmarks about their centroid (the square root of the above formulation is inadvertently omitted by Bookstein, 1991). Centroid size exhibits all the desirable properties of a size variable, in particular that of being uncorrelated with shape under a null hypothesis of no allometry (Mosimann, 1970; Bookstein, 1989a). In this study, centroid size was found to be positively correlated with standard length ( $r = 0.93$ ;  $P < 0.001$ ).

Shape coordinates allow the study of shape variation by considering triangles of landmarks. Two landmarks are chosen as baseline and are given coordinates [0,0] and [1,0], respectively. With a baseline AB (i.e., with A = [0,0] and B = [1,0]), the coordinates of C are then transformed to [X',Y'], following equations in Bookstein (1989b). This is repeated across the n-2 nonbaseline landmarks. The new coordinates [X',Y'] are termed shape coordinates, the properties of which are demonstrated by Bookstein

(1991:125–186). Of particular importance is that they encompass all possible shape variables derivable from interlandmark distances (i.e., ratios, angles, areas, etc.). As noted earlier, configurations of landmarks optimally superimposed using a least-squares procedure based on Procrustes-distances demonstrate greater statistical power than shape coordinates when used to test equity of shape amongst populations (Rohlf, 2000).

Shape coordinates in this study were derived using the baseline LM3-LM15 (Fig. 1) and applied in three subsequent analyses. The first examined variation within the six samples of *G. cypha*, whereas the second examined variation within the seven samples of *G. robusta*. Size variation in each of these analyses was first evaluated by using ANOVA to test for differences in centroid size among populations. Mean shapes were derived for populations of each species by first averaging then plotting shape coordinates.

Shape differences were evaluated among populations of each species by using MANOVA individually for each of the 23 nonbaseline coordinate pairs, then summarized using canonical variates analysis (CVA; Marcus, 1989). All morphological distances (Mahalanobis  $D^2$ ) values obtained from CVA were corrected for sample size using the formula in Marcus (1993). Classification results from CVAs of the shape coordinates were compared with those of 100 randomized runs using the dataset. Congruence between truss and shape-coordinate analyses was then assessed using Mantel tests of Mahalanobis distance matrices (as per Douglas and Endler, 1982).

The third analysis examined variation among all 13 populations (i.e., both species together) using relative warp analysis (RWA; Bookstein, 1991; Rohlf, 1993). To understand relative warp analysis, one must first understand the concept of a thin-plate spline (Bookstein, 1989b). Given two forms, one can use an interpolation function (i.e., the thin-plate spline) to map landmarks from one form [the “tangent configuration” (as per Rohlf et al., 1996)] onto corresponding landmarks of the second. In this sense, thin-plate splines are analogous to deformation grids of Thompson (Thompson, 1917). A shape-space (“bending energy”) metric expresses the level of deformation required to map the image to its target form. Put simply, RWA consists of fitting a thin-plate spline for the transformation of each specimen in the sample to a single tangent configuration. Variation among specimens is described in terms of the variance in the parameters of the spline function and are expressed relative to the bending

energy matrix. Relative warps are principal components of this space, and describe the major trends in shape variation within the overall sample (Rohlf, 1993). Various forms of RWA can be obtained by varying the exponential weight for the bending energy metric ( $\alpha$ ) between zero and one. When  $\alpha > 0$ , geometrically small-scale variation is given less weight than large scale variation. This has the effect of reducing the weight given to regions having more landmarks relative to that given to regions having fewer (and hence more widely spaced) landmarks. We used  $\alpha = 1$  because we were interested in morphometric differences at a larger scale. In addition, we were looking for shape variation in regions other than the nuchal hump, which comprised 40% (10/25) of our landmarks (Fig. 1). It also corresponds to the relative warp analysis of Bookstein (1989b, 1991; see Bookstein, 1996:146–147). As noted by Rohlf et al. (1996), the value of  $\alpha$  has no effect on most of the analyses employed herein because it merely scales the partial warps. The results of most multivariate analyses (e.g., MANOVA, CVA, for example) are scale free. For our analysis, mean shape-coordinate forms were calculated for all 13 samples, with the grand mean as the tangent configuration.

We also compared patterns of morphological shape variation in *G. robusta* and *G. cypha* by deriving taxonomic distances among the five sympatric populations only, based upon population means for relative warp axes one and two, derived from GLS-superimposed specimens. Both matrices were compared against a matrix of geographic distances derived from latitude-longitude values and against one another using two-way Mantel tests.

## RESULTS

*Body size and shape variation in Gila cypha.*—Centroid size varied significantly among populations ( $F_{5,142} = 7.33$ ;  $P < 0.0001$ ) with Little Colorado River specimens significantly larger than all other groups (Student-Neuman-Keuls test). All shape coordinate pairs showed significant differences among populations (all MANOVA,  $P < 0.0001$ ), and the overall MANOVA was also significant (Wilks'  $\Lambda = 0.002$ ;  $X^2_{240} = 743.47$ ;  $P < 0.0001$ ). Variation can be most clearly seen by examining shape coordinate plots among samples (Appendix 1). Three aspects are emphasized: shape of the nuchal hump; relative size of the head; and dimensions of the caudal peduncle. The nuchal hump is relatively more pronounced and anteriorly projected in Little Colorado River *G. cypha* (Appendix 1D), where-

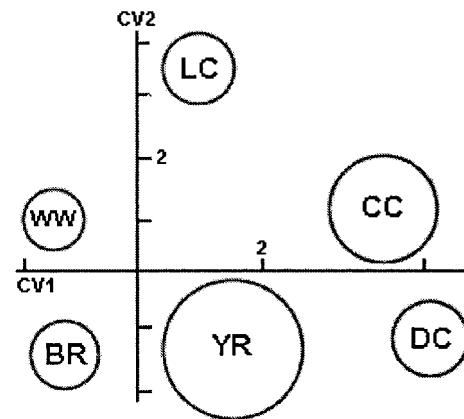


Fig. 2. Shape variation among six *Gila cypha* populations based on the first two variates derived from a canonical analysis of shape coordinates. Population abbreviations follow Table 1. Circles represent 95% confidence intervals for the group mean.

as specimens from Cataract Canyon (Appendix 1B) have a relatively slight hump. The position of the three landmarks bounding the operculum (LMs 16, 18 and 19; Fig. 1) indicate that specimens from Cataract Canyon have relatively larger heads than do those from the Little Colorado River, whereas all other populations are intermediate. Finally, there is significant variation in lengths and depths of the caudal peduncle, with specimens from Cataract Canyon having peduncles that are relatively shorter in length but that taper in depth from anterior to posterior, particularly when compared to those from Black Rocks Canyon (Appendix 1A), Westwater Canyon (Appendix 1E), or Yampa River (Appendix 1F). Desolation Canyon individuals (Appendix 1C) have peduncles that are uniformly deeper and nontapering than all others. Other aspects of shape variation are found in the distance from pectoral to pelvic fins (LMs 2–20), which is reduced in the Little Colorado River population. Fish from this population also have the longest anal fin base, which is reduced in specimens from Cataract Canyon. Also, Desolation Canyon individuals have shallowest body depths.

A canonical variate analysis of shape coordinates resulted in three statistically significant variates (of five), accounting for 95.9% of the among-population variation ( $P < 0.0001$ ; Fig. 2). Again, Little Colorado specimens were well separated from the other five populations. All Mahalanobis  $D^2$  distances were also significant ( $P < 0.0001$ ) and demonstrated a high degree of among-sample variation. Classification results were significantly different from chance, with

TABLE 2. CLASSIFICATION RESULTS FOR *Gila cypha* FROM A CANONICAL ANALYSIS OF SHAPE COORDINATES. Numbers in parentheses are classification results from truss analysis of McElroy and Douglas (1995). Key to sample codes is given in Table 1.

	BR	CC	DC	LC	WW	YR
BR	24 (24)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)
CC	0 (0)	11 (11)	0 (0)	0 (0)	0 (0)	0 (0)
DC	0 (0)	0 (0)	22 (22)	0 (0)	0 (0)	0 (0)
LC	0 (0)	0 (0)	0 (0)	28 (28)	0 (0)	0 (0)
WW	3 (1)	0 (0)	0 (0)	0 (1)	54 (55)	0 (0)
YR	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (5)

97.3% of the specimens being correctly allocated to their collection site (Table 2). This value is significantly different from that obtained from 100 randomized canonical variate analyses (mean of 65.5%;  $\sigma = 3.50$ ;  $P < 0.01$ ). Specimens from Westwater and Black Rocks, the two sites with smallest  $D^2$  values, also group closest to one another in canonical space (Fig. 2).

*Body size and shape variation in Gila robusta.*—Roundtail chub from Rifle and Debeque canyons were significantly larger than those from the other five populations but were indistinguishable from one another (Student-Neuman-Keuls test;  $F_{6,208} = 9.34$ ;  $P < 0.0001$ ). As with *G. cypha*, all shape coordinate pairs differed significantly among populations (all  $P < 0.0001$ ), and a significant MANOVA resulted (Wilks'  $\Lambda = 0.002$ ;  $X^2_{288} = 1154.9$ ;  $P < 0.0001$ ). Here, variation in peduncle and head characteristics is emphasized. Specimens from Cataract Canyon (Appendix 2B) have a relatively shorter peduncles that (again) taper in depth from anterior to posterior, whereas those from Desolation Canyon (Appendix 2C) are noticeably longer and of uniform depth. Debeque (Appendix 2F) and Rifle (not shown) Canyons have relatively smaller heads. Indeed, the latter two populations are characterized by having all landmarks of the head (with the exception of LMs 11, 13 and 25; Fig. 1) placed relatively more anteriorly when compared to other populations. Additional aspects of shape variation are found in relationships of body depth to fin placement. For example, Debeque and Rifle populations have deepest bodies yet shortest distances between pectoral and pelvic fins, whereas Westwater Canyon specimens seemingly have shallowest body depths. The development of a nuchal hump is slight in all *G. robusta* populations.

Three (of six) statistically significant canonical variates accounted for 85.3% of among-group variation (Fig. 3). As with *G. cypha*, all

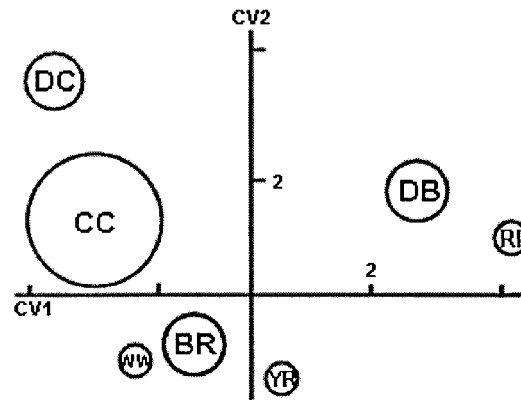


Fig. 3. Shape variation among seven *Gila robusta* populations based on the first two variates derived from a canonical analysis of shape coordinates. Population abbreviations follow Table 1. Circles represent 95% confidence intervals for the group mean.

Mahalanobis distances (and therefore all among-group comparisons) were significant ( $P < 0.0001$ ). Mahalanobis  $D^2$  values varied greatly. The lowest [i.e., 11.9 sdu (= standard deviation units)] occurred between Westwater and Black Rocks specimens, which were closest together in CV-space (Fig. 3). The highest value (76.5 sdu) occurred between Cataract and Rifle Canyons. Populations from Cataract and Desolation Canyons were well removed from the other five, with 96.3% of all specimens correctly allocated to site of origin (Table 3). This value is significantly different from that obtained from 100 randomized analyses (54.9%;  $\sigma = 3.42$ ;  $P < 0.01$ ).

*Body size and shape variation among all samples.*—As might be expected from the above, body size (as measured by centroid size), varied among all 13 samples. Two groups were detected ( $F_{12,350} = 7.94$ ;  $P < 0.0001$ ): One consisted of *G. robusta* from Rifle and Debeque Canyons and *G. cypha* from the Little Colorado; the second consisted of all other populations.

Relative warp analysis clearly separated populations of *G. cypha* from those of *G. robusta*, except for Desolation and Caratac Canyons (Fig. 4). Body shapes of *G. cypha* at the latter two locations were more *robusta*-like. Of all *cypha*-like forms, those from the Little Colorado River were most distinct, paralleling the results of the CVA of shape coordinates (Fig. 2).

Operational Taxonomic Units with low values on the first relative warp (RW1) clearly have a *cypha*-like morphology (i.e., deeper body with pronounced nuchal hump; smaller head; longer, narrower peduncle; Fig. 5A), whereas those



TABLE 3. CLASSIFICATION RESULTS FOR *Gila robusta* FROM A CANONICAL ANALYSIS OF SHAPE COORDINATES. Numbers in parentheses are classification results from truss analysis of McElroy and Douglas (1995). Key to sample codes is given in Table 1.

	BR	CC	DC	WW	YR	DB	RI
BR	19 (18)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
CC	0 (0)	6 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
DC	0 (0)	0 (0)	24 (24)	0 (0)	0 (0)	0 (0)	0 (0)
WW	2 (0)	0 (0)	0 (0)	52 (52)	2 (1)	0 (2)	0 (0)
YR	3 (1)	0 (0)	0 (0)	0 (0)	62 (63)	0 (1)	0 (0)
DB	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	20 (19)	0 (0)
RI	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	24 (24)

with higher values corresponded to a *robusta*-like form (i.e., larger head with no nuchal hump and broader peduncle; Fig. 5B). The second relative warp (RW2) is an axis of within-species variation. Here, decreasing values correspond to increasing prominence of the nuchal hump and concavity of the head. Little Colorado River *G. cypha* exhibit extreme *cypha*-like morphology when compared to specimens from Yampa River, Black Rocks, and Westwater Canyons. Variation in *G. robusta* is also exhibited along this axis, with atypical morphology (with respect to relative nuchal development) found in Cataract Canyon. Patterns of shape variation for each species (depicted in Fig. 4) are statistically congruent (Mantel  $Z = 10.834$ ;  $r = 0.721$ ;  $P = 0.02$ ).

*Congruence among truss and geometric analyses.*—McElroy and Douglas (1995) did not present Mahalanobis distance ( $D^2$ ) and classification

matrices for their truss analyses. These are presented in Tables 2 and 3. For *G. cypha*, good agreement exists between  $D^2$  values obtained from geometric and truss analyses (Mantel  $Z = 0.88$ ;  $t = 3.15$ ;  $P = 0.007$ ). The same significant relationship was observed for analyses of *G. robusta*. Therefore, both geometric and truss analyses support a single relative pattern of variation. In addition, classification matrices for geometric and truss analyses closely resemble one another (Tables 2–3). For *G. cypha*, the sole difference occurred at Westwater Canyon, where the truss analysis correctly reallocated 55 (of 57) specimens, whereas the shape coordinate analysis reallocated 54 specimens. Although numbers of specimens correctly reallocated are similar in both analyses of *G. robusta* (diagonal of Table 3), the placement of incorrectly allocated specimens differs, particularly for Westwater Canyon.

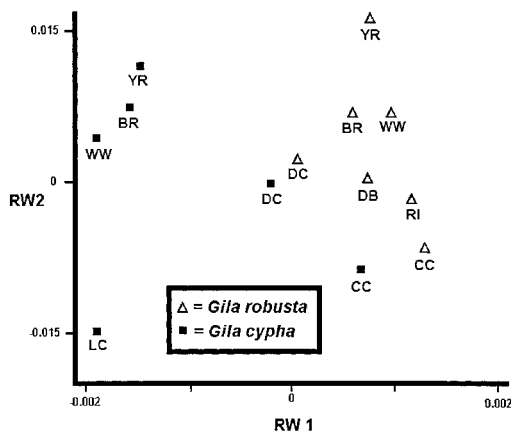


Fig. 4. Shape variation among 13 populations of *Gila cypha* and *Gila robusta* based upon the first two relative warps (RWs) of a relative warp analysis of shape coordinates using the grand mean as the tangent configuration. Population abbreviations follow Table 1.

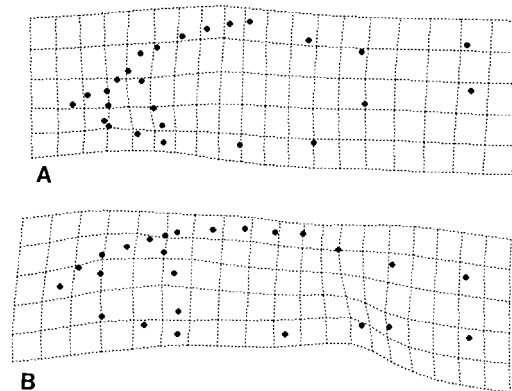


Fig. 5. Splines illustrating shape variation among the first relative warp axis of Figure 4. (A) corresponds to negative values of the warp and depicts a phenotype indicative of *Gila cypha*; (B) corresponds to positive values of the warp and demonstrates a *Gila robusta* phenotype.

## DISCUSSION

*Methodology.*—With  $\alpha = 1$  (i.e., large-scale patterns emphasized), the RWA still depicted the nuchal hump as an overriding factor in shape discrimination among populations of *G. cypha* and between it and *G. robusta*. This would be expected when only the two species were compared, because nuchal characteristics are paramount in discriminating among them (Douglas et al., 1998). However, subtle discrimination among populations of *G. cypha* was surprising and attests to the power of the geometric approach. Variation is also apparent in other body regions (Appendices 1–2), and these also contributed to discrimination.

*Previous research.*—Numerous studies have been undertaken to clarify the taxonomic confusion surrounding *G. robusta*, *G. elegans*, and *G. cypha*. Most relied upon pre-1960s Colorado River surveys (i.e., preimpoundment surveys; reviewed by Holden, 1991). Holden (1968) evaluated 309 individuals over 19 (of 35) characters. Specimens were: 130 *G. robusta*; 99 *G. elegans*; 16 *G. cypha*; 55 *G. elegans*  $\times$  *G. cypha*; and three *G. robusta*  $\times$  *G. elegans*. Holden concluded that *G. robusta* and *G. elegans* should be considered valid species. However, the relationship between *G. cypha* and *G. elegans* is clouded by the presence of what appeared to be intergrade forms. Specimens varied from typical *cypha* (i.e., abrupt hump and long snout) to typical *elegans* (i.e., smooth hump and no snout). Holden (1968) also concluded that collections to that time were not sufficiently large to clearly demonstrate morphological differences between the two forms. Apparent intergrades suggested either introgressive hybridization or phenotypic variability associated with environmental conditions.

Holden and Stalnaker (1975) also reported intergrades in the upper basin, whereas R. R. Miller argued these were actually *G. cypha* (T. W. Joseph, J. A. Shinning, R. J. Behnke, and P. B. Holden, U.S. EPA/U.S.F.W.S., 1977, unpubl.). Other important studies were reviewed by Douglas et al. (1989), Douglas (1993), and McElroy and Douglas (1995). Douglas et al. (1998) again demonstrated (as did Holden, 1968) that *G. robusta* was most easily separated from *G. cypha* and *G. elegans*.

*Present study.*—Differences with regard to overall body size are apparent within both *G. cypha* and *G. robusta*. In the former, the Little Colorado population has the largest individuals. Those from Desolation, Westwater, and Black Rocks

Canyons are intermediate in size, whereas fish from Yampa River and Cataract Canyon are smallest. For *G. robusta*, largest individuals are found in Rifle and Debeque Canyons, whereas smallest are again at Cataract Canyon. Remaining populations are intermediate in size.

Size-free shape coordinates tell a similar story: *G. cypha* from the Little Colorado River is most distinct, displaying classic humpback chub morphology (i.e., pronounced nuchal hump; small, concave head; thin caudal peduncle). However, Desolation and Cataract Canyon *G. cypha* show *robusta*-like morphology, with larger heads and reduced nuchal humps. Desolation Canyon *G. cypha* have longer and thicker peduncles irrespective of body size, whereas those from Cataract Canyon have more truncated peduncles. Both Desolation and Cataract Canyon *G. cypha* group closely with *G. robusta* from the same location when both species were included in the same analysis. However, Desolation and Cataract Canyon *G. cypha* also reflect differences in morphology when both were contrasted one against the other.

The unique morphology of Cataract Canyon *G. cypha* has been previously noted. R. A. Valdez (U.S. Bureau of Reclamation, 1988, unpubl.) observed that Cataract Canyon *G. cypha* were generally smaller fish as adults (i.e., < 200 mm TL), with blunt heads and overhung snouts, shallow nuchal humps, and scaleless napes and breasts (many of these characteristics were noted herein). Dorsal and anal ray counts of Cataract Canyon *G. cypha* were inconsistently 9 and 10, respectively (which are typical counts for the species). Valdez argued that genetic studies were needed to ascertain whether these fish were indeed *G. cypha*, because they certainly differed from the typical *G. cypha* phenotype. Our results (and those of McElroy and Douglas, 1995) suggest these fish (and those from Desolation Canyon) are indeed phenotypically different, appearing more *robusta*-like in their morphologies than do other *G. cypha* populations.

Another difficulty with the Cataract Canyon *G. cypha* population was noted by Karp and Tyus (1990), who stated that all upper basin *G. cypha* are presumed native except those in Cataract Canyon. The latter may be derived (at least in part) from a 1981 stocking of juvenile fish from Black Rocks. However, *G. cypha* from Cataract Canyon are phenotypically quite distinct from those at Black Rocks, particularly with regard to relative length and depth of head and relative length of caudal peduncle. This may indicate those *G. cypha* stocked in 1981 from Black Rocks did not survive to reproduce.

Failure of indigenous fishes to establish

themselves at a new location following translocation may be more common than originally suspected. For example, in an attempt to enhance whitefish biomass from an economic standpoint, whitefish (*Coregonus*) were stocked from other sources into Alpine lakes of Switzerland. In spite of multiple attempts with a variety of stocks from numerous lakes, genetic analyses employing microsatellite DNA indicated endemic *Coregonus* populations have indeed maintained themselves, with no genetic contribution from previous stocking attempts (Douglas and Brunner, 2001). Although relationships among forms cannot be determined in the absence of genetic analyses, one should not argue similarity among forms based upon historical stocking from one water source to another.

Shape variation in *G. robusta* is extensive. Larger (i.e., classic *robusta*-like) specimens from Rifle and Debeque Canyons actually display smaller heads for their size than do smaller fish from Cataract Canyon. Interestingly, individuals with shortest peduncles are from Cataract Canyon, followed by those from Rifle and Debeque Canyons, whereas specimens with longer peduncles are from Desolation Canyon. Cataract Canyon *G. robusta* also had thinnest caudal peduncles (a *cypha*-like trait), whereas those from Yampa River had thickest. Size of nuchal hump and depth of cranial concavity were reduced in all *G. robusta* examined.

*Truss versus geometric analyses.*—A quantitative comparison of the distance-based truss analysis versus the coordinate-based geometric analysis was nonsignificant, indicating that both provided the same level of resolution. This (more than likely) is a reflection of the fact that both studies shared 92% of their landmarks. Classification matrices from these studies were also congruent, again suggesting unanimity of results. However, the visualization of shape and shape change provided by the geometric analyses was superior, both within- and among-populations and species.

The necessity of developing bona fide visual cues for use by fishery managers and technicians to discriminate among species in the field has been discussed by Douglas et al. (1989). These researchers provided a series of qualitative characters to help resolve identification of *G. robusta* and *G. cypha* in the field. However, the application of geometric morphometrics (as in this study) provides classificatory cues at a broader and more visual level than does a qualitative evaluation. Thus, the use of this technique to quantify and visualize morphological differences among populations and species of

endangered and threatened fishes is not only supported but recommended.

It is also noteworthy that a location effect was documented with regard to phenotypic variation in both species. Not only are populations of each species distinct among themselves, but both species display statistically similar patterns of geographic variation that are themselves correlated with latitude/longitude.

*Hybridization.*—Hybridization has always been an undocumented spectre with regard to upper basin *Gila*. Valdez and Clemmer (1982) argued that apparent hybridization between *G. cypha* and *G. robusta* may have resulted from riverine impoundments and water development projects that altered the Colorado River hydrograph. Historic flow regimes were perceived by these researchers (and others) as an important reproductive isolating mechanism (discussed in Douglas and Douglas, 2000). Depletion of peak flows and possibly temperature alterations may have altered niches of these species such that they now overlap extensively, and thus hybridize. Similarly, in the Yampa River, chronic depletion of historic peak flows has been hypothesized as a possible limiting factor for reproduction of *G. cypha* (H. M. Tyus and C. A. Carp, USFWS Report, 1989, unpubl.). Karp and Tyus (1990) also noted that reproductive *G. cypha* and *G. robusta* were sympatric within eddy habitats during the five-to-six week period following highest spring runoff in the Yampa River, again suggesting the potential for hybridization. Kaeding et al. (1990) noted that spawning of these two species at Black Rocks also overlapped temporally. However, radio-tagged *G. robusta* displayed a wider dispersion during breeding season (max. displacement = 33.9 km;  $n = 17$ ) than did more sedentary *G. cypha* (max. displacement = 1.4 km;  $n = 33$ ).

“Studies to clarify the taxonomy of the *Gila robusta* complex and of *Gila cypha* should be continued throughout the entire Colorado River Basin. Concurrent studies of the ecological requirements and genetic variability among the various biological entities of *Gila* are needed to provide information essential to the design of programs to save the rarer forms from extinction” (R. H. Kramer, Utah Coop FWS unit, Utah State Univ., 1967, unpubl.). Although this directive sounds amazingly contemporary, it was stated over 33 years ago. Yet, researchers are seemingly no closer to unraveling the taxonomic confusion within *Gila*. Hybridization has been documented in formation of upper basin species (DeMarais et al., 1992) and in distribution of large-scale (allozymic) variation in many low-



er basin populations (Dowling and DeMarais, 1993). Although morphological analyses can designate individuals to species or groups, these techniques alone cannot unambiguously ascertain the degree of introgression exhibited by individuals or populations or even if hybridization has occurred. Instead, morphology can discriminate individuals once the range of genetic variance has been determined (as in Sofield et al., 1984). A morphological range of variation has been established for upper basin *Gila* (McElroy and Douglas, 1995; this study). All that remains is for these results to be linked with those from genetic analyses. Only then will questions about morphological intermediacy of populations and species be unambiguously determined.

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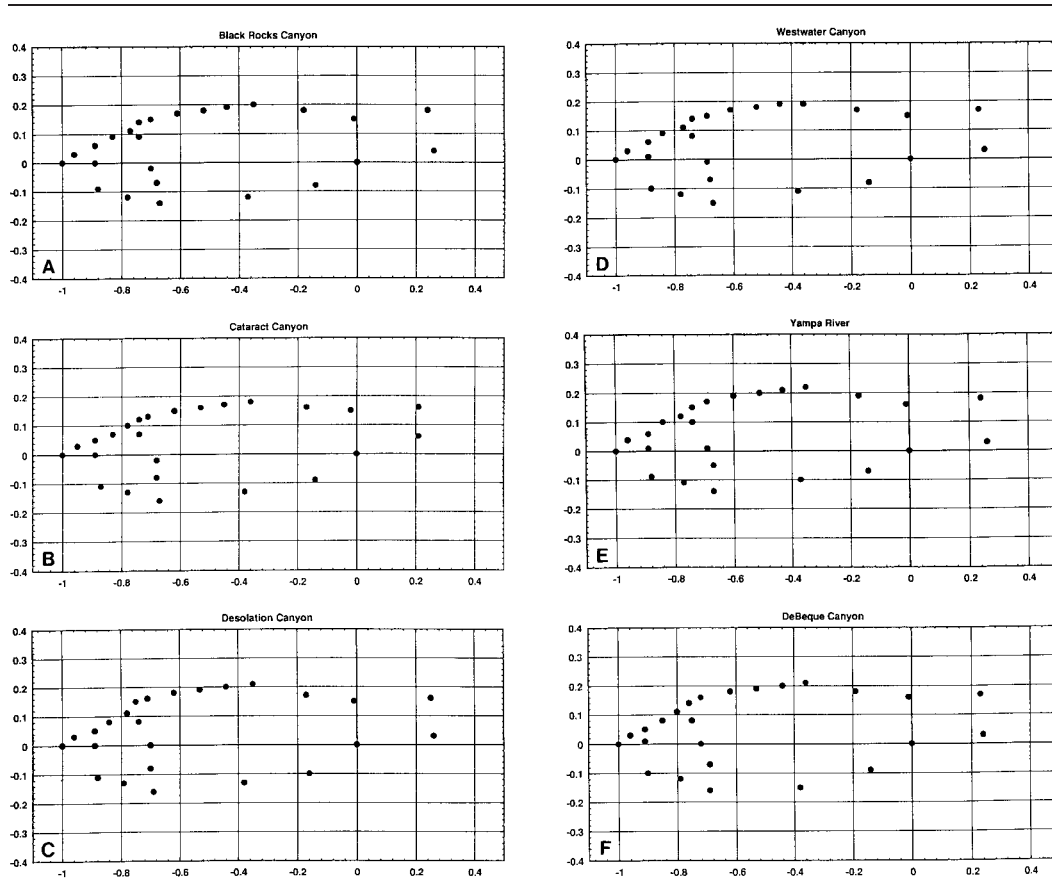
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APPENDIX 1. MEAN BODY SHAPES FOR SIX POPULATIONS OF *Gila cypha*.



APPENDIX 2. MEAN BODY SHAPES FOR SIX (OF SEVEN) *Gila robusta* POPULATIONS.