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Redescription, Geographic Variation, and Taxonomic Status of Rio Grande Silvery Minnow, *Hybognathus amarus* (Girard, 1856)

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Hybognathus amarus **is redescribed and geographic variation assessed to resolve its taxonomic status.** ***Hybognathus amarus*** **is distinguished from congeners by its small size, ovate cross-section, short basioccipital with a wide and shallowly concave posterior margin, moderate orbit diameter that is less than gape width or snout length, rounded snout, subterminal mouth, lateral band that does not intersect the lateral line, and relatively short intestine. Characters and univariate and multivariate analyses of morphometric variables support recognition of** ***H. amarus*** **as a valid taxon but did not support designation of subspecies for** ***H. amarus*** **from the Rio Grande, New Mexico; the Pecos River, New Mexico; or the lower Rio Grande, Texas. Rather, most geographic variation was at the scale of subsamples within those regions. Comparisons of body size, orbit diameter, gape and body width, body circumferential scale counts, and basioccipital process shape useful for identification of all** ***Hybognathus*** **species are presented. Conservation measures are needed to ensure survival of the formerly widespread and common** ***H. amarus*****, since it presently occurs only in the middle Rio Grande, New Mexico, which is < 10% of its original range.**

SYSTEMATICS of most species in the cyprinid genus *Hybognathus* are confused in large part because of morphological similarities among mainly allopatric forms. At least 15 species or subspecies have been described (e.g., Girard, 1856; Cope and Yarrow, 1875; Hildebrand, 1932), but only seven are currently recognized (Robins et al., 1991). Systematics of only *H. hayi* (Jordan, 1885b; Fingerman and Suttkus, 1961) and *H. hankinsoni* (C. L. Hubbs in Jordan, 1929) have remained stable.

The Rio Grande silvery minnow, *H. amarus*, typifies the complicated systematic history of most *Hybognathus* species. Originally described as *Algoma amara* (Girard, 1856) from the Rio Grande near Brownsville, Texas, it and *H. placitus* were placed in synonymy with *H. nuchalis* (Jordan, 1885a; Hubbs and Ortenburger, 1929; Bailey, 1956). Hubbs and Ortenburger (1929) and Jordan (1929), however, believed *H. placitus* a valid taxon. Hubbs (1940), Koster (1957), and Trevino-Robinson (1959) subsequently treated Rio Grande *Hybognathus* as *H. placita amara*. Based on morphological differences, Koster (1957) distinguished Canadian River basin *Hybognathus* (*H. placitus*) from Rio Grande *H. p. amara*. Bailey (1956), however, submerged *H. placitus* within *H. nuchalis*, stating that it was an ecophenotype of the latter. Differences in the basioccipital process among several *Hybognathus* justified resurrection of *H. placitus* as distinct (Niazi and Moore, 1962; Bailey and Allum, 1962; Al-Rawi and Cross, 1964) and similarities in the process allied Rio Grande *Hybognathus* with *H. nuchalis*.

In a comprehensive review, Pflieger (1971)

separated *H. nuchalis* into Atlantic Slope *H. regius* and Missouri River drainage *H. argyritis*, while restricting *H. nuchalis* to the Mississippi River and Gulf Coastal drainages. Pflieger (1980) suggested that the nominal Rio Grande form, *H. amarus*, was separable from *H. nuchalis*, a view supported by Hlohowskyj et al. (1989) who discovered differences in pharyngeal filtering apparati among *Hybognathus* species. Cook et al. (1992) found fixed allozyme differences at each of two loci that differentiated Rio Grande *Hybognathus* from *H. nuchalis* (Sod-2, Est-3) and *H. placitus* (Est-1, Est-3). Phylogenetic studies by Cavender and Coburn (1988), Mayden (1989), and Schmidt (1994) of *Hybognathus* further justified recognition of *H. amarus* (Smith and Miller, 1986; Robins et al., 1991). Sublette et al. (1990) noted that a comprehensive morphological study of *H. amarus* was lacking.

The type locality of *H. amarus* is near Brownsville, Texas, the extreme southern extent of its range and, as a consequence, does not reflect possible intraspecific variation of *H. amarus*. The importance of defining morphometric variation and clarifying the taxonomic status of *H. amarus* is heightened by recent and dramatic reductions of this once widespread and abundant species (Bestgen and Platania, 1991). Past collections have documented *H. amarus* from three main areas: the Rio Grande in New Mexico, Pecos River in New Mexico, and the Rio Grande downstream of the Pecos River confluence in Texas/Mexico (Bestgen and Platania, 1991). Small collections from intervening river reaches (Big Bend on the Rio Grande and the Pecos River in Texas) substantiate its wide historic occurrence. Presently, the species inhabits only a 300-km reach (< 10% of its former range) of the Rio Grande in New Mexico between Cochiti and Elephant Butte reservoirs. Reductions in distribution and abundance prompted listing of *H. amarus* as endangered by the New Mexico Department of Game and Fish, and the species is listed as endangered by the US Fish and Wildlife Service (1994). Our purpose here is to provide a complete morphometric and meristic description of *H. amarus*, analyze intraspecific variation, and resolve its taxonomic status.

Methods

Morphometric and meristic data collection techniques followed Hubbs and Lagler (1964) and Chernoff et al. (1982) except for modifications detailed below. Dorsal-fin and pelvic-fin lateral-line measurements were the vertical distance between origin of each fin and the lateral line. Orbit diameter was the greatest horizontal distance between the fleshy rims. Length of the basioccipital was from the pharyngeal pad to the most posterior projection, and widths were measured just posterior to the pharyngeal pad (basal width) and at the posterior margin. All basioccipital process measurements were made with a dissecting microscope and ocular micrometer or with calipers and a microscope. Circumferential scale counts above and below the lateral line were made two scale rows anterior to dorsal fin; total body and caudal peduncle circumferential scale counts were the sum of each location pair plus two lateral-line scales. Proportions were derived by dividing raw measurements by standard length (SL). Institutional abbreviations follow Leviton et al. (1985).

Most specimens of Rio Grande *Hybognathus* used in this study were from the Rio Grande in New Mexico (RGNM), the Pecos River in New Mexico (PRNM), and the Rio Grande in Texas downstream of the Pecos River confluence (RGTX). Small samples from the Rio Grande near Big Bend and the Pecos River in Texas were not statistically different than those from the RGTX and were combined. Other *Hybognathus* species were represented mostly by specimens from single collections although *H. placitus* and *H. nuchalis* were from two and four localities, respectively.

Statistical analyses were conducted using Statistical Analysis Systems statistical software (SAS Institute, 1988). Morphometric variables were log transformed. Sample sizes of specimens from RGNM, PRNM, and RGTX were about equal. A random subset of 16 males and 24 females, from which all measurements were taken, was used to characterize sexual dimorphism. Analysis of covariance (ANCOVA, SAS PROC GLM) was used to analyze each measurement; log SL was the covariate. Use of a covariate removed morphometric variable variation associated with overall body size, allowing a more equitable comparison across groups (i.e., sexes, regions, and species). The ANCOVA assumption of parallel regression lines among groups was tested, and only variables that met this assumption were compared. Untransformed meristic variables were subjected to analysis of variance (ANOVA). When overall F-tests were significant, group differences were determined by least-squares means procedures. The large number of univariate variables evaluated warranted use of the Bonferroni correction, where the probability value for acceptance of a significant difference was 0.05 divided by the number of comparisons (Harris, 1975).

Intraspecific variation in *H. amarus* was eval-

uated by univariate and multivariate comparisons of morphometric and meristic variables for specimens from RGNM, PRNM, and RGTX. Principal components analysis (PCA, SAS PROC PRINCOMP) of meristic (correlation matrices) variables and sheared PCA of morphometric variables (Bookstein et al., 1985; algorithm of L. Marcus modified by M. E. Douglas) were used to determine intraspecific variation in *H. amarus*. Multiple group PCA (Douglas, 1993) was not conducted because within-group covariance matrices were significantly different.

Stepwise discriminant analysis (PROC STEPDISC, stepwise procedure, $P = 0.15$ significance level for variable entry into model) was used to identify a subset of meristic and morphometric variables for each analysis. Multicollinearity of variables was reduced by setting a tolerance limit of 0.05 (Affifi and Clark, 1990) which eliminated from consideration variables correlated at ≥ 0.95. A discriminant classification function (SAS PROC DISCRIM), based on the variable subset, determined classification rates for specimens from different regions. Covariance matrices were not equal among regions, so within-region covariance matrices and quadratic functions were used. The CROSSVALIDATE option (a jackknife resubstitution) was used to test discrimination ability of the function (SAS Institute, 1988).

To test whether grouping specimens a priori into the arbitrary RGNM, PRNM, and RGTX regions was appropriate, we subdivided specimens from those regions into three subsamples each. The RGNM and PRNM subsamples of 30 specimens each were from single collections. Due to a paucity of specimens, the three RGTX samples were composites of two or more collections: a Pecos River, Texas, collection was combined with three collections from the Rio Grande downstream of its confluence with the Pecos River to near Laredo (n = 22 specimens); two collections from near Brownsville were combined (n = 22); and the Rio Grande Big Bend sample was a composite of five collections (n = 13). Thus, nine subsamples and 237 specimens were used, and discriminant analysis classified individual specimens to subsamples. Presumably, if classification rates of specimens to subsamples approached classification rates achieved for the three arbitrary regions, then regions may not be the appropriate scale to examine intraspecific variation in *H. amarus*.

Univariate comparisons of *H. amarus* were limited to *H. placitus* and *H. nuchalis*, species with which *H. amarus* has been previously confused. Interspecific differences among seven *Hybognathus* species were analyzed with multivariate techniques, with emphasis on comparisons of *H. amarus* with other species.

Intraspecific variation in morphology of the basioccipital process of *H. amarus* was examined by region (i.e., RGNM, PRNM, and RGTX). Intra- and interspecific comparisons (30 specimens per species, except 15 per each *H. hayi* and *H. hankinsoni*) were analyzed by ANCOVA (log-SL was covariate) and least-squares means tests. Principal components and discriminant function classification analyses were also conducted.

Osteological characters of eight disarticulated or cleared-and-stained specimens of *H. amarus* from the Rio Grande (n = 6) and Pecos River (n = 2), New Mexico, were compared with one specimen of *H. nuchalis* from the Red River, Oklahoma, and one from the Buffalo River, Mississippi.

Hybognathus specimens collected from the Pecos River since 1938 were examined to estimate when *H. placitus* was introduced and whether there was morphological evidence of hybridization. Uni- and multivariate techniques (PCA, DFA) were used to compare morphometric, meristic, and basioccipital process data from known pure specimens of *H. amarus* and *H. placitus* and potential hybrid specimens. Putative hybrid specimens were segregated prior to analysis based on comparison of variables and overall appearance.

Results

Hybognathus amarus (Girard 1856)
Rio Grande silvery minnow

Diagnosis.—A small species of *Hybognathus* restricted to warmwater reaches of the Rio Grande drainage that is distinguished from congeners by the following traits: body subterete, relatively heavy, round to ovate in cross-section; basioccipital short and deflected ventrally, with shallowly concave posterior margin; orbit diameter (0.053 × SL) much less than gape width or snout length; snout rounded, overhangs upper lip from ventral aspect; subterminal mouth extends horizontally to just short of the anterior margin of the orbit. Lateral band rests on but does not intersect lateral line on caudal peduncle. Pharyngeal filtering apparatus includes a broad pharynx and short, stubby papillae on the pharynx and basibranchial (Hlohowskyj et al., 1989). Intestine relatively short (4.7 × SL ± 0.70). Unique alleles at loci Est-1, Est-3, and Sod-2 distinguish *H. amarus* from *H. nuchalis*, *H. placitus*, and *H. hankinsoni* (Cook et al., 1992).

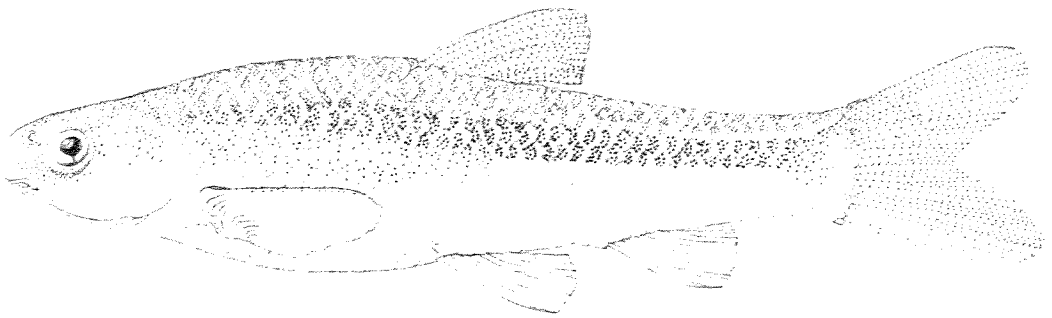

Fig. 1. *Hybognathus amarus*, adult male, 60.2 mm standard length.

Description.—General features of the physiognomy and pigmentation (Fig. 1) and selected osteological features (Fig. 2) of *H. amarus* are illustrated; proportional measurements and frequency distributions of selected meristic variables for *H. amarus*, *H. nuchalis*, and *H. placitus* are summarized in Tables 1–4. Dorsal-fin rays 7 (n = 6), 8 (278), or 9 (1); anal-fin rays 7 (32) or 8 (263); pectoral-fin rays 14 (10), 15 (21), 16 (17), 17 (3), or 18 (1); pelvic-fin rays 8 (51) or 9 (1); principal caudal-fin rays 15 (1), 17 (1), 18 (8), 19 (278), or 20 (7). Gill rakers on first arch 9 (1), 10 (5), 11 (10), 12 (6), 13 (3), 14 (1), or 15 (2). Preoperculomandibular pores 9 (5), 10 (35), 11 (14), or 12 (7). Pharyngeal teeth usually 0,4–4,0 (21), less commonly 0,5–4,0 (3), or 0,4–5,0 (5); teeth in excess of 0,4–4,0 arrangement usually not firmly attached. Teeth relatively long with expanded and flattened grinding surfaces. Intestine tightly coiled counterclockwise (from ventral aspect). Body fully scaled, although scales slightly embedded and smaller on breast. Scales as high as wide and round except ventrally, which are pointed posteriorly.

Fins of *H. amarus* moderate in length and variable in shape. Specimens from RGNM have dorsal and pectoral fins nearly always rounded at tips and straight at distal margin whereas PRNM and RGTX specimens more often pointed, slightly longer, and sometimes have slightly falcate distal margins. Pectoral fins of males flare broadly from base to a triangular fan shape, qualitatively appear as long as wide, flattened at the distal margin, and those of breeding males have thickened rays. Pectoral fins of females shorter, narrower, oval-shaped, about twice as long as wide, more rounded at the distal margin, and have slender rays. Pelvic fins of males sometimes longer than those of females and flattened at posterior margin.

Pigmentation.—Freshly preserved specimens light greenish-yellow dorsally fading to light cream or white ventrally; lateral band pale. Older preserved specimens darker yellow-brown or tan dorsally; narrow dorsal midline gold to dark-brown. Lateral coloration yellow-tan to cream, below lateral line cream with yellowish suffusion to near white ventrally. Specimens from turbid water pallid in life and in preservation.

Light to dark lateral band, about one scale wide, originates from a diffuse triangle at caudal base and extends forward, arches upward anterior to dorsal-fin insertion, and tapers to point just behind head. Lateral band above and does not intersect lateral line. Lateral band dark and broad posteriorly to dorsal-fin insertion and light and narrow anteriorly.

Few melanophores posterior to pelvic-fin insertion and ventral to lateral line and all proximal to lateral line except on caudal triangle. Anterior to pelvic insertion, a few melanophores ventral to and near lateral line; venter otherwise unpigmented. Scales in first row below lateral line and anterior to pelvic-fin insertion may be faintly outlined by melanophores, especially dorsally. Scales above lateral line sometimes outlined in a diamond pattern. Head and snout pigmentation moderately dense and extending laterally over the cheek and snout to about the middle of the eye. Tip of snout lightly or not pigmented. Upper and lower lips and ventral surface of head immaculate.

Anal- and pelvic-fin bases and interradial integument immaculate. Pectoral-fin melanophores variable, usually on rays 1–4 (range 1–9), and darkest on leading ray. Dorsal-fin rays pigmented, integument between rays 1 and 6 has a few melanophores. Caudal-fin rays with melanophores but none on membranes. Life colors and pigmentation as above but lighter.

Tuberculation.—Small, fine tubercles common anterodorsally and laterally in nuptial males and females. Tubercles densely distributed over dorsal and lateral surfaces of the snout and head

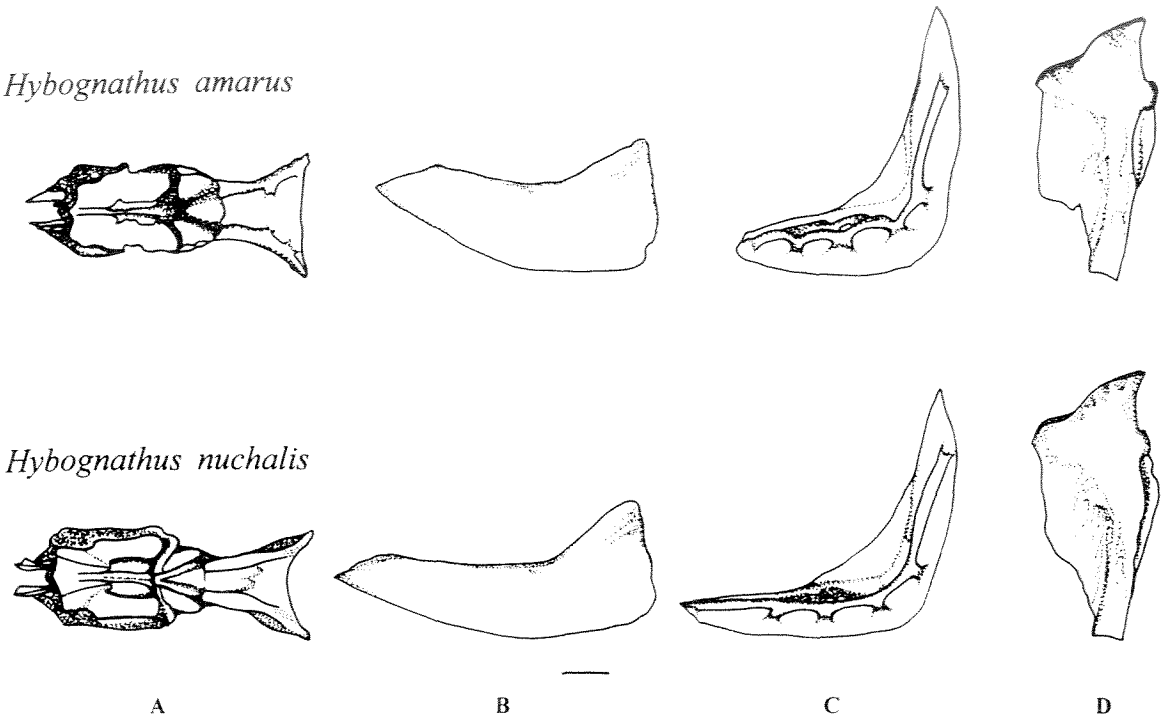

Fig. 2. Dorsal view of the pharyngeal process of the basioccipital (A, posterior margin on right) and lateral view of the left interopercle (B), preopercle (C), and hyomandibula (D) of *Hybognathus amarus* (59.6 mm standard length, top), and *H. nuchalis* (54.5 mm, bottom). A 1 mm scale bar is shown at bottom.

and extend to posterior margin of operculum. Tubercles on head retrorse or erect. Smaller, less densely spaced tubercles present ventrally over the isthmus and branchiostegal rays. On individual scales, a single, evenly spaced row of 14–20 slightly retrorse tubercles lines the posterior margin. Smaller tubercles distributed randomly over the scale surface.

Minute tubercles on fin rays of median fins. Each branch of individual fin rays with one or two rows of tubercles extending to near the distal tip. Tubercles associated with each paired fin but much more common on pectoral fins. Tuberculation dense on leading edge and upper surface of pectoral fin rays. Tubercles most dense near base of fin rays where blocks of four to six tubercles in two or three rows are associated with each ray segment. Tubercle rows divide at fin-ray branches and extend distally in single row to margin of fin ray. Tubercles similar on pelvic fins but less dense and, except for the two outside rays, arranged in a single row. In females, tubercles are less dense, smaller, and less evident on pectoral fins.

Sexual dimorphism.—Significant sexual dimorphism was found for body depth, distance from pelvic-fin origin to lateral line, and pectoral-fin length. Significance of the first two variables was probably due to the expanded body cavity of some ripe female specimens. Pectoral-fin length as proportion SL was longer in males ($\bar{x}$ = 0.208, 0.197–0.224) than females ($\bar{x}$ = 0.180, 0.166–0.203); pectoral-fin shape differences were previously described. Sex of 27 of 30 nonreproductive specimens (90%) was correctly identified using pectoral-fin size and fin shape differences. Sexual dimorphism was not noted for any meristic variables.

Intraspecific variation.—Univariate comparisons of morphometric variables of specimens from RGNM, PRNM, and RGTX revealed that only body depth, pelvic-fin origin–lateral-line distance, caudal-peduncle least depth, bony interorbital distance, and upper jaw length met the equality of slopes requirement of ANCOVA. Body depth was significantly different only between RGNM and PRNM, whereas pelvic-fin origin–lateral-line distance and caudal-peduncle depth were significantly different between RGNM and both PRNM and RGTX. Bony interorbital distance was significantly different only for RGTX and PRNM. Upper jaw length was not significantly different among regions.

Univariate analyses of 13 meristic variables (pharyngeal tooth, gill raker, pectoral- and pelvic-fin ray, and preopercular pore counts ex-

TABLE 1. MEAN, STANDARD DEVIATION (SD), AND RANGE OF PROPORTIONS (VARIABLE/STANDARD LENGTH* 1000) OF MORPHOMETRIC VARIABLES FOR SPECIMENS OF *Hybognathus amarus* FROM RIO GRANDE DRAINAGE, NEW MEXICO, TEXAS, AND MEXICO, COMPARED TO *H. nuchalis* AND *H. placitus*. Significant differences ($P \leq 0.01$) were determined by analysis of covariance and least-squares means and are denoted (*) for the species and measurement that are different from *H. amarus*.

Measurement	*Hybognathus amarus* (n = 256) Mean (SD) Range	*Hybognathus nuchalis* (n = 58) Mean (SD) Range	*Hybognathus* placitus (n = 60) Mean (SD) Range
Standard length	58.5 (11.93) 30.5–82.5	66.8 (17.68) 44.5–102.2	61.2 (8.57) 40.7–82.7
Dorsal origin–snout	526 (15.50) 481–592	510 (12.88)* 473–536	508 (15.13)* 436–550
Dorsal origin–caudal base	511 (18.60) 463–643	523 (10.53)* 500–550	526 (13.36)* 499–567
Dorsal origin–occiput	324 (17.52) 268–368	302 (10.50)* 276–329	294 (14.78)* 265–339
Pelvic origin–snout	538 (17.32) 484–610	522 (9.96)* 501–542	529 (12.28)* 490–565
Anal origin–snout	736 (16.59) 676–841	735 (13.71) 701–762	725 (12.23)* 687–753
Anal origin–caudal base	284 (15.61) 243–371	285 (12.43) 255–321	285 (14.28) 254–317
Dorsal origin–anal origin	309 (14.57) 278–369	322 (13.56)* 299–351	308 (11.18) 277–334
Body depth	248 (18.55) 201–310	245 (11.49) 216–269	234 (13.98)* 204–273
Body width	152 (17.15) 107–208	139 (10.43)* 114–164	157 (13.10) 121–185
Dorsal origin–lateral line	140 (8.75) 107–184	143 (5.85) 127–156	126 (8.22)* 108–141
Pelvic origin–lateral line	108 (12.55) 80–147	109 (8.52) 87–130	99 (12.32)* 81–133
Caudal-peduncle length	194 (12.98) 155–265	194 (7.86) 175–211	197 (11.17) 174–231
Caudal-peduncle depth	110 (6.06) 94–131	109 (6.45) 96–123	104 (5.85)* 87–116
Caudal-peduncle width	44 (9.51) 19–68	44 (8.11) 26–60	35 (9.31)* 18–52
Head length	245 (14.68) 197–285	248 (11.77)* 219–271	238 (20.48) 203–280
Head depth, occiput	166 (7.47) 149–191	163 (8.57) 148–179	162 (6.75) 146–189
Head depth, eye	123 (8.26) 102–145	121 (8.39) 106–138	115 (8.04)* 100–130
Head width	141 (6.77) 127–167	128 (6.89)* 116–142	142 (5.31) 128–155
Interorbital, fleshy	94 (5.76) 79–116	86 (4.96)* 69–98	89 (6.62)* 72–103
Interorbital, bony	84 (4.83) 67–100	78 (3.39)* 68–85	80 (3.83)* 69–89
Snout length	74 (5.31) 55–88	77 (4.72)* 66–87	78 (4.63)* 69–94
Orbit diameter	53 (7.48) 38–75	58 (6.70)* 47–73	46 (3.78)* 38–55
Upper jaw length	56 (5.41) 41–71	56 (4.44) 44–64	56 (5.21) 44–67
Gape width	65 (4.16) 53–80	58 (4.06)* 50–65	69 (3.85)* 62–77

TABLE 1. CONTINUED.

Measurement	*Hybognathus amarus* (n = 256) Mean (SD) Range	*Hybognathus nuchalis* (n = 58) Mean (SD) Range	*Hybognathus placitus* (n = 60) Mean (SD) Range
Dorsal-fin length	216 (14.63) 181–254	219 (10.63)* 196–242	216 (14.39) 182–264
Anal-fin length	160 (11.84) 129–191	155 (9.57) 131–178	153 (10.08)* 132–175
Pectoral-fin length	202 (17.62) 149–250	196 (11.83)* 164–224	196 (16.72) 175–272
Pelvic-fin length	149 (10.49) 123–208	155 (7.52)* 140–178	140 (6.72)* 124–156
Basioccipital length[a]	42 (5.75) 36–59	47 (6.50)* 38–64	49 (5.25)* 39–60
Basioccipital basal width[a]	22 (2.75) 17–28	22 (4.50) 15–33	11 (2.50)* 7–17
Basioccipital posterior margin width[a]	35 (4.80) 26–45	39 (8.25) 21–54	12 (2.75)* 6–17

[a] Basioccipital process measurements for n = 31 specimens per species.

cluded) revealed differences among regions (significant overall F-tests) for all except dorsal- and caudal-fin ray counts. Least-squares means tests for the 11 remaining variables showed seven significant differences between RGNM and RGTX, 10 between RGNM and PRNM, and 11 between RGTX and PRNM.

Plots of principal component scores for meristic variables (not shown) showed broad overlap among the three regions. The best separation of specimens to regions was provided by plots of scores of sheared principal components II and III for morphometric variables (Fig. 3). Dorsal origin–caudal base, dorsal origin–occiput, and pelvic origin–snout variables contributed most to the minimal separation of groups along the sheared PCA II axis (Table 5).

Discriminant analysis performed on lateral line, predorsal, total body circumference, total caudal peduncle, and caudal peduncle above lateral-line scale variables correctly classified 77% (RGNM), 78% (RGTX), and 67% (PRNM) of the specimens. For each region, classification errors were distributed about equally among the other two regions. Morphometric variables (pelvic-fin origin–snout, body depth, caudal-peduncle length, head width, orbit diameter, and pectoral- and pelvic-fin lengths) subjected to discriminant analysis yielded classification rates of 93% (RGNM), 94% (PRNM), and 83% (RGTX). Classification errors were equally distributed among regions.

Individuals of *H. amarus* were correctly classified to subsamples (n = 9) an average of 40% (20–75%) of the time with meristic variables. Morphometric variable classification rates averaged 81% (67–100%) for samples from RGNM and PRNM that were composed of single collections but were only 40% (31–50%) for samples from RGTX that were composed of multiple collections.

Univariate ANCOVA for basioccipital measurements of *H. amarus* from different regions indicated parallel slopes for basal and posterior

TABLE 2. LATERAL-LINE SCALE COUNTS OF *Hybognathus amarus* FROM THREE GEOGRAPHIC REGIONS AND FOR *H. nuchalis* AND *H. placitus*.

Species	34	35	36	37	38	39	40	41	42	$\bar{x}$	SD
Hybognathus amarus											
Rio Grande, NM		5	28	39	16	7	2				
Pecos R., NM			3	14	55	14	4				
Rio Grande, TX	5	25	35	31	2						
Hybognathus amarus (total)	5	30	66	84	73	21	6			37.0	1.22
Hybognathus nuchalis	1	15	20	16	4	2				36.2	1.08
Hybognathus placitus		1	1	14	17	15	10	1	1	38.4	1.29

TABLE 3. PREDORSAL SCALE COUNTS OF *Hybognathus amarus* FROM THREE GEOGRAPHIC REGIONS AND FOR *H. nuchalis* AND *H. placitus*.

Species	12	13	14	15	16	17	18	19	$\bar{x}$	SD
Hybognathus amarus										
Rio Grande, NM			5	33	43	18	7	1		
Pecos R., NM	1	1	16	47	18	6	1			
lower Rio Grande, TX	1	8	64	23	2					
H. amarus (total)	2	9	85	103	63	24	8	1	15.1	1.12
Hybognathus nuchalis	1	15	36	4		1	1		13.9	0.94
Hybognathus placitus			6	13	15	17	8	1	16.2	1.26

margin widths but not for length. No significant differences were found.

Intraspecific variation among osteological characters of *H. amarus* from RGNM and PRNM was not noted. No specimens from RGTX were examined for osteological characters.

Comparisons with H. nuchalis and H. placitus.— Characters useful for distinguishing all *Hybognathus* species are presented (Table 6). We focused on detailed comparisons of *H. amarus* with *H. nuchalis* and *H. placitus*, species with which the former has been previously confused.

Observations indicated *H. nuchalis* was larger and more deep-bodied and laterally compressed than *H. amarus*. The snout of *H. nuchalis* was sharper, more wedge-shaped, and from ventral view overhung the upper lip less. A line extending horizontally backward from the tip of the upper lip intersected the eye in *H. nuchalis*, whereas the line was below the eye in *H. amarus*. The lateral band of *H. nuchalis* was less distinct and intersected the lateral line on the caudal peduncle. Scale outline was more distinctly diamond-shaped, with some melanophores distributed ventrally to the lateral line on the caudal peduncle, and the snout and upper lip were heavily pigmented (pigment sometimes on terminus of lower jaw).

The posterior margin of the basioccipital *H. nuchalis* was generally more deeply notched producing prongs rather than the shallow, nearly emarginate concavity of *H. amarus* (Fig. 2; see also figs. 18 and 21 in Niazi and Moore, 1962). The preopercle of *H. nuchalis* was less robust; lower limb was longer and pointed anteriorly; and the interopercle was shorter, less deep, and less massive than *H. amarus* (Fig. 2). The ventral edge of anterior wing of the hyomandibula in *H. nuchalis* sloped backward rather than being sharply truncate as in *H. amarus*.

Least-squares means comparisons showed significantly shorter dorsal origin–snout, dorsal origin–occiput, pelvic origin–snout, and pelvic-fin lengths. Significantly narrower body, head, fleshy interorbital, bony interorbital, and gape widths in *H. nuchalis* than *H. amarus* (Table 1) are consistent with the relatively more laterally compressed morphology of the former species. Conversely, *H. nuchalis* had greater dorsal origin–caudal base, dorsal origin–anal origin, snout, dorsal-fin, and pelvic-fin lengths. Mean orbit diameter and intestine length (9.2 × SL vs 4.7 × SL) of *H. nuchalis* was also greater. Qualitatively, mean gape width/mean orbit diameter ratio was unity in *H. nuchalis* (0.058/0.058) but greater in *H. amarus* (0.065/0.053). *Hybognathus nuchalis* had significantly fewer lateral line (median 36 vs 37), predorsal (14 vs 15), and body circumference below lateral line (14 vs 15) and total (26 vs 28 or 29) scales than *H. amarus*.

Observations indicated body conformation of *H. placitus*, although similar to *H. amarus*, was more streamlined, slender, ventrally flattened and had an arched dorsal profile. Orbit diameter was smaller. The head was longer and wedge-shaped (in lateral view) and the snout more pointed in comparison to the blunt and rounded head and snout of *H. amarus*. Dorsal and pectoral fins were sometimes pointed and falcate, although some PRNM and RGTX *H. amarus* showed such characteristics. The basioccipital process of *H. placitus* was long, narrow, and peglike, and without a broadly expanded posterior margin (fig. 8 in Niazi and Moore, 1962; compare figs. 72 and 76 in Sublette et al. 1990).

Hybognathus placitus had significantly shorter dorsal-fin origin–snout and occiput, pelvic- and anal-fin origins–snout, dorsal- and pelvic-fin origin–lateral-line distances, shorter anal- and pelvic-fin lengths, smaller body depth, bony and fleshy interorbital widths, and orbit diameter (Table 1). However, *H. placitus* had significantly greater dorsal-fin origin–caudal-fin base distance, snout length, and gape width. Qualitatively, mean upper jaw length/mean orbit diameter ratio for *H. placitus* was greater than unity (0.056/0.046) whereas that of *H. amarus*

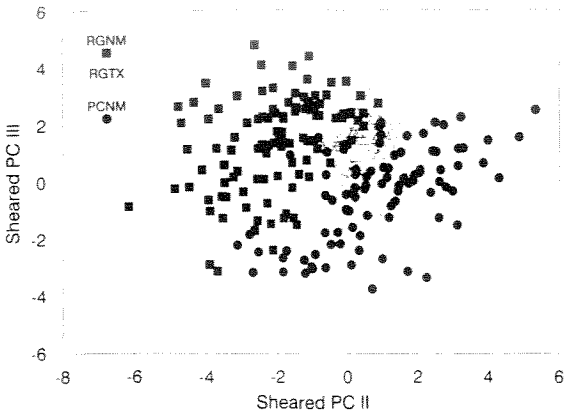

Fig. 3. Plot of scores from sheared principal components (PC) II and III for 28 morphometric variables for *Hybognathus amarus* from the Rio Grande, New Mexico (RGNM), lower Rio Grande, Texas (RGTX), and the Pecos River, New Mexico (PRNM).

was near unity (0.056/0.053). *Hybognathus placitus* had significantly more scales than *H. amarus* for lateral line (38 vs 37), predorsal (16 vs 15), body circumference above and below lateral line and total (14 vs 12, 16 vs 15, and 32 vs 28 or 29, respectively), and total caudal peduncle circumference (16 vs 14) counts.

Comparisons with all Hybognathus species.—Principal components analysis of meristic variables (not shown) showed broad overlap in variation among most *Hybognathus* species. The *H. amarus* cluster almost completely encompassed all other species. *Hybognathus hankinsoni* and *H. argyritis* were distinctly separated along PC II, and both were nearly separated from *H. hayi*, *H. placitus*, and *H. regius* along PC III.

Plots of scores from sheared PC II and III for morphometric variables showed that *H. amarus* clustered with *H. argyritis*, *H. nuchalis*, and *H. placitus* along sheared PC II (Fig. 4). Dorsal origin–and anal origin–caudal base and dorsal origin–occiput variables loaded most heavily on sheared PC II whereas body depth, dorsal origin–anal origin and anal origin–caudal base variables loaded most heavily on sheared PC III (Table 5). Each of the four species in that cluster has relatively small dorsal origin– and anal origin–caudal base measurements and relatively long dorsal origin–occiput measurements (Table 1, in part). *Hybognathus hankinsoni*, *H. regius*, and *H. hayi* were nearly separate from *H. amarus* along PC II, and from each other along PC II or III.

Discriminant function classification analysis of variables total body circumference scales and separate counts above and below the lateral line,

TABLE 4. BODY CIRCUMFERENCE SCALE COUNTS OF *Hybognathus amarus* FROM THREE GEOGRAPHIC REGIONS AND FOR *H. nuchalis* AND *H. placitus*.

Species	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	$\bar{x}$	SD
Hybognathus amarus																	
Rio Grande, NM					9	3	24	18	19	14	15	5					
Pecos R., NM				1	8	18	18	19	18	4	3	1					
Rio Grande, TX	1		2	11	47	20	13	4									
H. amarus (total)	1		2	12	64	41	55	41	37	18	18	6				28.2	2.01
Hybognathus nuchalis				5	28	12	12	1								26.6	0.97
Hybognathus placitus							3	2	14	12	8	6	12	2	1	31.7	1.92

TABLE 5. COEFFICIENTS OF SHEARED PRINCIPAL COMPONENTS (PC) II AND III FOR ANALYSIS OF INTRASPECIFIC VARIATION IN *Hybognathus amarus* FROM THE RIO GRANDE, NEW MEXICO, RIO GRANDE, TEXAS, AND PECOS RIVER, NEW MEXICO (FIG. 3), AND FOR ANALYSIS OF INTERSPECIFIC VARIATION AMONG *H. amarus*, *H. argyritis*, *H. hankinsoni*, *H. hayi*, *H. nuchalis*, *H. placitus*, AND *H. regius* (FIG. 4).

Measurement	*Hybognathus amarus*		All *Hybognathus* species	
	PC II	PC III	PC II	PC III
Standard length	0.068	0.027	0.181	−0.125
Dorsal origin–snout	−0.320	0.050	−0.294	−0.154
Dorsal origin–caudal base	0.518	−0.064	0.466	0.159
Dorsal origin–occiput	−0.365	0.013	−0.381	−0.219
Pelvic origin–snout	−0.358	0.034	−0.284	−0.206
Anal origin–snout	−0.254	−0.177	−0.220	0.073
Anal origin–caudal base	0.272	0.259	0.391	−0.231
Dorsal origin–anal origin	0.210	−0.284	0.066	0.463
Body depth	0.173	−0.326	−0.087	0.565
Body width	−0.037	−0.422	−0.224	0.161
Dorsal origin–lateral line	0.096	−0.096	−0.002	0.243
Pelvic origin–lateral line	0.134	−0.203	−0.032	0.262
Caudal-peduncle length	0.279	0.096	0.327	−0.121
Caudal-peduncle depth	0.078	−0.006	−0.003	0.111
Head length	0.064	0.128	0.076	−0.010
Head depth, occiput	0.044	−0.015	−0.012	0.091
Head depth, eye	0.065	0.059	0.024	0.042
Head width	0.033	−0.055	−0.109	0.031
Interorbital, fleshy	0.009	0.008	−0.051	−0.013
Interorbital, bony	0.006	0.009	−0.041	−0.005
Snout length	−0.034	−0.001	−0.014	0.005
Orbit diameter	0.055	0.050	0.096	0.025
Upper jaw length	0.020	0.032	−0.019	0.017
Gape width	−0.011	0.015	−0.049	−0.040
Dorsal-fin length	0.021	0.380	0.142	−0.116
Anal-fin length	−0.045	0.281	0.067	−0.174
Pectoral-fin length	0.135	0.428	−0.002	−0.119
Pelvic-fin length	−0.035	0.188	0.079	−0.024

total caudal-peduncle scales and those below the lateral line, and predorsal-scale rows correctly classified an average of 57% (28–90%) of the specimens. Only 28% of *H. amarus* specimens were correctly classified; other specimens were misclassified as each of the other species, but most often (41%) as *H. nuchalis*. Discriminant function classification analysis of morphometric variables upper jaw length, fleshy interorbital width, caudal peduncle least depth, pelvic-fin–lateral line distance, orbit diameter, gape width, head length, anal-fin length, caudal-peduncle length, and dorsal-fin origin–snout distance classified an average of 94.5% of *H. amarus* correctly (Table 7). *Hybognathus amarus* was most often misclassified (4%) as *H. placitus*. *Hybognathus argyritis* (93%), *H. hankinsoni* (94%), *H. nuchalis* (93%), and *H. placitus* (95%) were correctly classified about as frequently as *H. amarus*.

Least-squares means of basioccipital length for *H. amarus* was significantly different from all species except *H. hayi*. Basal and posterior margin basioccipital widths of *H. amarus* were significantly different from all other species except *H. nuchalis*. However, qualitative differences between *H. amarus* and *H. nuchalis* in the posterior margin of the process (previously described) generally distinguish each species.

The PCA and pharyngeal process measurements for all *Hybognathus* species (Fig. 5, Tables 1, 6, in part) suggested separation of species into four groups. One group had a long basioccipital with a relatively narrow posterior margin (*H. placitus*), and the other three groups were characterized by a short basioccipital with a posterior margin that was either narrow (*H. hankinsoni*), intermediate (*H. argyritis*, *H. hayi*, *H. regius*), or relatively wide (*H. amarus*, *H. nuchalis*). Discriminant function analysis of basioccipital measurements of seven *Hybognathus* species correctly classified only 60% of the specimens.

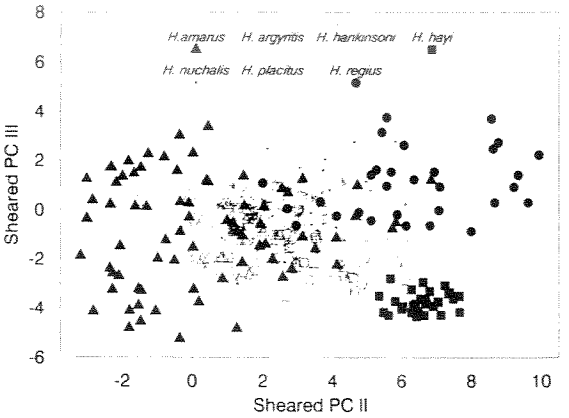

Fig. 4. Plot of scores from sheared principal components (PC) II and III for 28 morphometric variables for seven *Hybognathus* species. Many *H. amarus* individuals whose scores were located near the centers of the *H. nuchalis* and *H. placitus* clusters were not plotted to increase clarity.

Hybridization.—Two possible hybrid specimens were found in a 1964 collection of fishes from the Pecos River (ASU 1308). High total body circumference scale counts of 31 and 33 initially indicated pure *H. placitus*, but the morphometric measurements, snout shape, and dorsal taper indicated hybrid origin and necessitated reexamination. Other specimens of *Hybognathus* from the same collection appeared to be pure *H. amarus*.

Scatter plots of meristic PCA scores were intermingled among species and putative hybrids; no discernible clusters were noted. Unlike meristic data, individual morphometric variables and scatter plots of morphometric PCA scores indicated that putative hybrid specimens clustered with *H. amarus*.

Discriminant analysis of meristic variables (lateral-line scales, predorsal-scale rows, scales

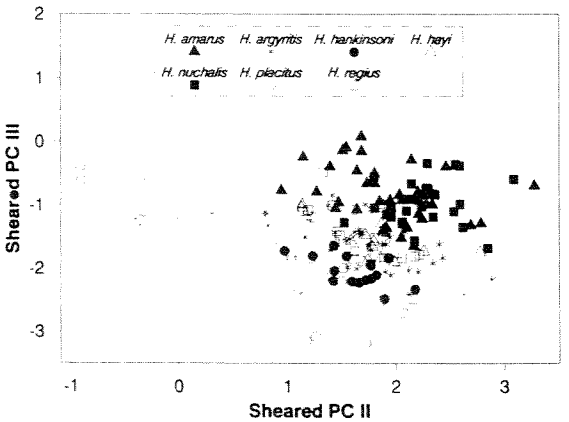

Fig. 5. Plot of scores from sheared principal components (PC) II and III for three pharyngeal apparatus measurements for seven *Hybognathus* species.

TABLE 6. SUMMARY OF QUANTITATIVE AND QUALITATIVE DIFFERENCES AMONG SEVEN SPECIES OF *Hybognathus*.

Character	*amarus*	*argyritis*	*hankinsoni*	*hayi*	*nuchalis*	*placitus*	*regius*
Maximum body size[a]	small	large	small	medium	large	large	large
Orbit diameter[b]	medium	small	medium	large	med.-large	small	med.-large
Gape width[c]	wide	moderate	moderate	narrow	narrow	wide	narrow
Body width[d]	round	ovate	ovate	compressed	compressed	round	compressed
Circumferential body scales[e]	medium	medium	high	low	low	high	medium
Basioccipital process[f]	wide	moderate	narrow	moderate	wide	narrow	moderate

[a] Data from specimens examined and from general literature sources: small ≤ 80 mm SL; medium = 80–100 mm SL; large ≥ 100 mm SL. Typical adult body sizes are usually much smaller.
[b] Mean eye diameter (% SL) from specimens examined in this study: small = 4.5–4.8% SL; medium = 5.3–5.6% SL; large = 5.8–7.0% SL.
[c] Mean gape width (% SL) from specimens examined in this study: narrow = 5.5–5.9% SL; moderate = 6.1–6.3% SL; large = 6.5–6.9% SL.
[d] Body shape and width (BW) measurements: round = round cross-sectional profile, BW 15–16% SL; ovate = oval profile slightly laterally compressed, BW 14–14.5% SL; compressed = laterally compressed, 11–13% SL.
[e] Median body circumferential scales: low = 26; medium = 27–29; high = ≥31.
[f] Width of posterior margin or basioccipital: wide = expanded and spatulate, 3.5–4.0% SL; narrow = peg or rod-shaped, 1.2–1.9% SL; moderate = slightly expanded posterior margin, 2.3–2.7% SL. Comparison of pharyngeal process of *H. argyritis* and *H. nuchalis* in Pflieger (1971).

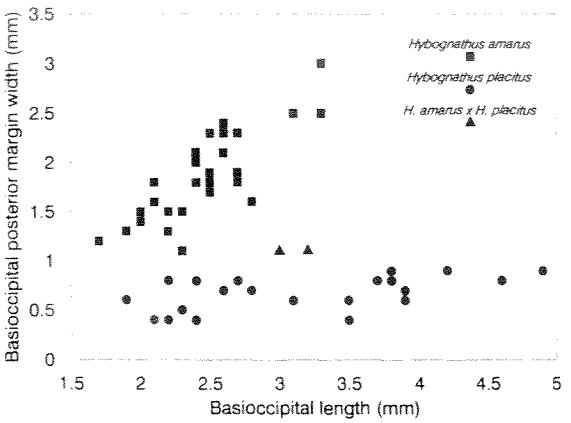

Fig. 6. Bivariate plot of basioccipital length and basioccipital posterior margin width for *Hybognathus amarus*, *H. placitus*, and two putative *H. amarus* × *H. placitus* hybrids.

above lateral line, body circumference scales above lateral line, caudal-peduncle scales below lateral line, and total caudal-peduncle scales) correctly classified *H. amarus* and *H. placitus* 91% and 90% of the time, respectively. One putative hybrid was classified as *H. amarus* and the other as *H. placitus*. Discriminant analysis of morphometric variables anal-fin origin–snout distance, dorsal-fin origin–occiput distance, gape width, body depth, dorsal-fin origin–lateral line distance, head length, snout length, orbit diameter, and pelvic-fin length correctly classified 95% of *H. amarus* and 97% of *H. placitus* specimens. Both putative hybrids were classified as *H. amarus*.

Principal components analysis (not shown) and plots of data (Fig. 6) suggested intermediate basioccipital posterior margin width and basioccipital length for putative hybrids compared to pure *H. amarus* and *H. placitus*. Discriminant function analysis of pharyngeal process measurements correctly classified all pure parental types and grouped putative hybrids with *H. placitus*.

Discussion

Systematics of *H. amarus* were confused historically, in large part because of unquantified intraspecific variation and morphological similarity to other *Hybognathus* species. Comparisons among species revealed that the small maximum body size, rounded body cross-section, moderate orbit diameter and body circumferential scale count, wide gape width, and differences in the basioccipital process distinguish *H. amarus* from congeners (Table 6). Our analyses of meristic, morphometric, and osteological characteristics combined with previous investigations (Pflieger, 1980; Hlohowskyj et al., 1989; Cook et al., 1992) provide strong evidence confirming *H. amarus* as a valid species.

Hybognathus amarus displays little sexual dimorphism in morphometric or meristic variables. Only pectoral-fin length is a reliable segregating character. Other statistically significant dimorphic differences are reliable only when specimens were reproductively ripe. Because sexual dimorphism detected by univariate analyses in *H. amarus* was limited to pectoral-fin length, sexes were combined in further analyses.

Intraspecific variation in *H. amarus* was investigated to determine whether designation of other taxa or subspecies was warranted. Although univariate comparisons of morphometric and meristic variables indicated differences among the three geographic regions within its historic range, no consistent affinity pattern (positive or negative) was noted between region pairs.

Principal component analyses of meristic and morphometric variables and pharyngeal process measurements did not provide good separation of specimens from different geographic regions. Alternatively, discriminant analysis classified specimens of *H. amarus* to appropriate geographic regions at moderately high rates for meristic variables and at high rates for morphometric variables.

Table 7. Summary of Discriminant Function Classification Analysis for Morphometric Variables for Seven Species of *Hybognathus*.

	n	*amarus*	*argyritis*	*hankinsoni*	*hayi*	*nuchalis*	*placitus*	*regius*
H. amarus	256	94.5	0.4	0.4		0.4	4.3	
H. argyritis	61	6.6	93.4					
H. hankinsoni	31	6.5		93.6				
H. hayi	30				100.0			
H. nuchalis	58	1.7		1.7		93.1		3.5
H. placitus	60	5.0					95.0	
H. regius	30					10.0		90.0

However, moderate (meristic variables) to high (morphometric variables) classification rates were also achieved when specimens were classified to six RGNM and PRNM subsamples of specimens from single collections. Low classification rates for RGTX specimens to three subsamples is likely the product of combining two or more collections taken at different times and places. Significant intraspecific variation in *H. amarus* was expressed among subsamples from a region, thereby reducing the importance of differences perceived among regions. The ambiguous results obtained from uni- and multivariate analyses of morphometric and meristic variables do not support recognition of subspecies of *H. amarus*.

Principal component analysis indicated broad overlap of some *Hybognathus* species in meristic and morphometric variables and emphasized their superficial similarity. High overlap between *H. amarus* and other *Hybognathus* species was probably due, in part, to the larger sample size and the greater geographic coverage for this species. Variation of *H. amarus* was characterized from 256 specimens from throughout its historic range whereas ≤ 30 specimens from one or two localities were used to characterize other *Hybognathus*. Although discriminant classification analysis indicated broad overlap of meristic variables, classification rates were high when based upon morphometric variables.

Uni- and multivariate analyses of the basioccipital process demonstrated generally consistent differences among *Hybognathus* species. The deeper concavity of the posterior margin of the process generally distinguishes *H. nuchalis* and *H. amarus*, but we, as well as Schmidt (1994), have noted some variation in the degree of emargination so this character should be used with others to differentiate the two species.

The rapid disappearance of *H. amarus* in the Pecos River is perplexing given the wide distribution and abundance of this species. Evidence of the introduction and establishment of *H. placitus* was first obtained from hybrid specimens collected from near Fort Sumner in 1964 (ASU 1308). By the mid-1970s, no *H. amarus* remained in the Pecos River and *H. placitus* occupied all reaches formerly inhabited by *H. amarus* (Cowley, 1979; Sublette et al., 1990). Cook et al. (1992) reported genetic evidence, wherein alleles unique to *H. amarus* were found in five of 20 specimens of *H. placitus* from the Pecos River, to support hybridization and genetic swamping as part of the cause for elimination of *H. amarus* from the Pecos River.

Reasons for the extirpation of *H. amarus* from the lower Rio Grande (as well as the Big Bend area) are more ambiguous. Previously, the last reported *H. amarus* from that reach (n = 1) was in 1961 (Bestgen and Platania 1991), but reexamination of that specimen revealed it was *H. placitus*. Thus, the last pure *H. amarus* from the lower Rio Grande were collected in the late 1950s (Trevino-Robinson, 1959; Edwards and Contreras-Balderas, 1991). The few specimens available from the lower Rio Grande during this time did not indicate hybridization was involved in extirpation of *H. amarus*.

Extirpation of *H. amarus* from much of its historic range has probably involved additional factors (Propst et al., 1987; Bestgen and Platania, 1990; 1991). Negative interactions with introduced fishes, including *H. placitus*, dewatering of stream reaches during critical life-history stages (e.g., spawning) or degraded water quality, and range fragmentation by reservoirs and irrigation diversion dams probably had locally varying influences on the elimination of *H. amarus* from most of its historic range. Continued existence of *H. amarus* in a short reach of the Rio Grande in central New Mexico is threatened by continued water development, habitat modification, contaminants, and introduced fishes. Immediate conservation efforts are needed to secure *H. amarus* in its remaining range and to restore it to larger portions of its historic range.

Material Examined

Hybognathus amarus, New Mexico (NM): Rio Chama; MSB 1163, Abiquiu (n = 2); Rio Grande; MSB 1135 San Ildefonso (n = 6), MSB 1132, Angostura Div. (n = 30), MSB 1171, Albuquerque (n = 15), MSB 1122, Albuquerque (n = 15), MSB 7489, Los Lunas (n = 30), MSB 1142, Las Cruces (n = 1), MSB 1148, Las Cruces (n = 3), MSB 1196, Las Cruces (n = 5), OKSU 5428, Albuquerque, (n = 46), Pecos River; ASU 1308, Fort Sumner (n = 116), KU 8362 (n = 28), KU 8318 (n = 35), KU (n = 40), Roswell, KU 8070, Lake McMillan, (n = 7), MSB 1161, Santa Rosa, (n = 30), MSB 1170, Fort Sumner (n = 30), MSB 1128, Roswell (n = 30), MSB 2636, Roswell, (n = 2), Texas (TX): Rio Grande; OKSU 11852 (n = 2), OKSU 5491 (n = 2), Big Bend, TNHC 4365, Castolon, (n = 2), TNHC 4545, S. of Terlingua Ck. (n = 1), TNHC 4660, Roma, (n = 17), TNHC 4778, Laredo (n = 6), TNHC 4786, Brownsville (n = 15), UMMZ 170193, Zapata (n = 19), UMMZ 170205, Brownsville (n = 98), Tornillo Ck.; UMMZ 127342, Big Bend (n = 3), Terlingua Ck.; UMMZ 159110 Big Bend (n = 4), Pecos R.; UMMZ 170115, Shumla (n = 14), UMMZ

89485, Fort Stockton, (n = 9). *Hybognathus argyritis*, South Dakota: Little Missouri R., UMMZ 178957, Camp Crook (n = 33), Nebraska (NB): Little Nemaha R., ZM 1225 (n = 30). *Hybognathus hankinsoni*, Colorado: South Platte R., Larimer-Weld canal, MSB 4806 (n = 31), NB: Battlecreek ZM 2076 (n = 30), Elkhorn R. ZM 2102 (n = 30). *Hybognathus hayi*, Florida: Escambia R., UMMZ 165033, Cantonment (n = 30), Illinois (IL): Little Muddy R., UMMZ 163019, DuBois, (n = 32). *Hybognathus nuchalis*, IL: Big Muddy Ck. MSB 1165 (n = 3); Oklahoma: Red R., MSB 4675 (n = 15), Mississippi: Tombigbee R., BSFC 1377 (n = 10), Tennessee: Ish Ck., UMMZ 200511 (n = 30). *Hybognathus placitus*, NB: S. Little Nemaha R., (n = 30), Platte R., (n = 30), NM: Ute Cr., MSB 1168, (n = 15), Revuelto Ck. MSB 4666 (n = 30), Pecos R., MSB 9120, Fort Sumner (n = 15). *Hybognathus regius*, New Jersey and Delaware: Delaware R., MSB 4674 (n = 30).

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