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Allozymic Divergence and Systematics of the Rio Grande Silvery Minnow, *Hybognathus amarus* (Teleostei: Cyprinidae)

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***Hybognathus amarus*, a cyprinid endemic to the Rio Grande drainage, has had a confused taxonomic and systematic history. We examined allozymic variation for 22 presumptive gene loci for two populations each of *H. amarus*, *H. hankinsoni*, and *H. placitus* and one population of *H. nuchalis*. *Dionda episcopa*, *Pimephales promelas*, and *Campostoma anomalum* were included as outgroups. Nei's (1978) genetic distance values were greater than 0.111 for all species pairwise comparisons in this study; intraspecific comparisons yielded genetic distances less than 0.005. Phenetic and phylogenetic analyses corroborated the hypothesis that *H. amarus* is a valid taxon, separate from *H. nuchalis* and *H. placitus* with which it was previously included.**

THE fishes of the cyprinid genus *Hybognathus* have been taxonomically perplexing almost since the description of the genus and *H. nuchalis* by Agassiz (1855). At least 15 forms have been proposed as species of *Hybognathus* (e.g., Girard, 1856; Cope and Yarrow, 1875; Hildebrand, 1932), but only seven are currently recognized as valid (Pfleiger, 1971; Robins et al., 1980; Smith and Miller, 1986). Among the latter, only *H. hayi* (Jordan, 1885a) and *H. hankinsoni* (C. L. Hubbs in Jordan, 1929; Bailey, 1954) have remained taxonomically stable since their original description. The other five forms (*H. amarus*, *H. argyritis*, *H. nuchalis*, *H. placitus*, and *H. regius*) have been variously arranged as subspecies of *H. nuchalis*. Much of the confusion was due to overall morphological similarity and lack of a comprehensive study of the genus (Burr, 1980).

The taxonomic history of the Rio Grande form, *Hybognathus amarus*, parallels that of several other members of the genus. Following its description as *Algoma amara* (Girard, 1856), it and *H. placitus* were placed in synonymy with *H. nuchalis* (Jordan, 1885a, 1885b; Hubbs and Ortenburger, 1929; Bailey, 1969) on the basis of similarities in body shape, eye size, fin shape, and scale structure. Jordan (1929) and Hubbs and Ortenburger (1929), however, considered *H. placitus* a valid taxon and Hubbs (1940), Koster (1957), and Trevino-Robinson (1959) subsequently treated the Rio Grande form of *Hybognathus* as subspecies of *H. placitus*.

The discovery by Niazi and Moore (1962) of distinctive differences in the shape of the pharyngeal processes of the basioccipital justified the removal of *H. placitus* from synonymy with *H. nuchalis*. Thereafter, Rio Grande *Hybognathus*, as well as Atlantic Slope and Missouri River forms (*H. regius* and *H. argyritis*, respectively), were considered subspecies of *H. nuchalis* until Pfleiger (1971) reviewed the taxonomy of the nominal forms. He resurrected *H. argyritis* and *H. regius* and suggested that the Rio Grande form of *Hybognathus* was separable from the Mississippi River form, *H. nuchalis*.

Since that time, others (e.g., Smith and Miller, 1986; Hlohowskyj et al., 1989) have followed Pfeiger (1971) in recognizing *H. amarus* as a valid taxon. Although efforts have been made to more accurately define several species of *Hybognathus* (Burr and Mayden, 1982; Hlohowskyj et al., 1989; Schmidt, 1989) and clarify the phylogenetic relationships within the genus and with related genera (Cavender and Coburn, 1988; Mayden, 1989; Schmidt, 1989), no comprehensive investigation has been conducted on the Rio Grande form, *H. amarus*.

Impetus to clarify the taxonomic status of *H. amarus* is heightened by its disappearance throughout much of its native range. Formerly, this species was widespread in the Rio Grande drainage (including the Pecos River) in New Mexico and Texas (Koster, 1957; Trevino-Robinson, 1959). Since the 1960s, however, it has declined dramatically throughout its range and is now present only in the middle Rio Grande of New Mexico (Bestgen and Platania, 1991).

Much of the decline of *H. amarus* may be attributed to modification of stream discharge

patterns and channel desiccation by impoundments, water diversion for agriculture, and stream channelization. The role of introduced fish species in the decline of *H. amarus* is not clear, but the establishment of *H. placitus* in the Pecos River in New Mexico coincided with the extirpation of *H. amarus* (Bestgen et al., 1989; Sublette et al., 1990).

In this investigation, we tested conflicting taxonomic and systematic hypotheses regarding relationships of *H. amarus*. Specifically, we examined the genetic relationships of Rio Grande *Hybognathus* with other species with which it has been placed in synonymy (*H. nuchalis* and *H. placitus*) and a taxonomically stable member of the genus, *H. hankinsoni*. We also explored the phylogenetic relationships of these species of *Hybognathus* and the presumably related cyprinid genera *Campostoma, Dionda*, and *Pimephales*, as well as investigated the possibility that introgressive hybridization occurred between native *H. amarus* and introduced *H. placitus* in the Pecos River, New Mexico.

Methods and Materials

Fishes were collected by seining and then transported to the laboratory for processing. Samples of skeletal muscle were dissected from each individual and immediately frozen in liquid nitrogen or in an ultracold freezer. Following dissection, the head of each specimen, as well as additional whole voucher specimens, were deposited in the Museum of Southwestern Biology (Division of Fishes), University of New Mexico (MSB).

Muscle samples were homogenized in an equal volume of grinding solution (EDTA buffered) and centrifuged at 20,000 *g* at 4 C for 20 min, and the supernatant was stored at −70 C. Because of high genetic variation found among and within cyprinid species (Buth, 1984), two different samples (Richardson et al., 1986) of all species of *Hybognathus* (except *H. nuchalis*) were assayed to permit evaluation of levels of intraspecific and interspecific genetic variation. Specimens from an introduced population of *H. placitus* in the Pecos River, New Mexico, were examined to determine whether there had been any genetic introgression with recently extirpated *H. amarus*. Specimens of *Campostoma, Dionda*, and *Pimephales* were used as outgroups for the phylogenetic analyses (Watrous and Wheeler, 1981).

Genetic variability was assayed using starch gel electrophoretic methods modified from Selander et al. (1971) and Harris and Hopkinson (1976). Alleles at each locus were assigned alphabetic designations with the most anodally migrating electromorph labelled as allele a. Gene products of presumptive enzyme loci were assayed for each specimen without knowledge of the individual's taxonomic affinity. Enzymes studied and buffer systems used are listed in Table 1.

BIOSYS-1 (Swofford and Selander, 1981) was used to calculate average number of alleles per locus (A), percent polymorphism (P), expected heterozygosity (H-exp), observed heterozygosity (H-obs), and coefficients of genetic distance

Table 1. Enzymes (Enzyme Commission Numbers in Parentheses), Loci, and Electrophoretic Conditions.*

Enzyme (EC number)	Locus	Electrophoretic conditions
Aspartate aminotransferase (EC 2.6.1.1)	mAat-A	A
	sAat-A	A
Esterase (EC 3.1.1.1)	Est-1	B
	Est-3	B
	Est-4	B
General protein (nonspecific)	Prot-1	C
	Prot-2	C
Glycerol-3-phosphate dehydrogenase (EC 1.1.1.8)	G3pdh-A	C
Isocitrate dehydrogenase (EC 1.1.1.42)	mIcdh-A	A
Lactate dehydrogenase (EC 1.1.1.27)	Ldh-A	A
	Ldh-B	A
Malate dehydrogenase (EC 1.1.1.37)	sMdh-A	A
	sMdh-B	A
Peptidase A (EC 3.4.13.11)	Pep-1	B
	Pep-2	B
	Pep-3	B
Phosphoglucomutase (EC 5.4.2.2)	Pgm-A	C
Phosphogluconate dehydrogenase (EC 1.1.1.44)	Pgdh-A	C
Superoxide dismutase (EC 1.15.1.1)	Sod-1	C
	Sod-2	C
	Sod-3	C
Xanthine dehydrogenase (EC 1.2.1.37)	Xdh-A	A

* A: Tris citrate pH 6.7/6.3, 75 ma/5 h; B: PG1-phosphate pH 6.7, 75 ma/7 h; C: Acid citrate pH 6.1/6.0, 75 ma/3 h.

TABLE 2. GENOTYPIC ARRAY FOR 22 LOCI IN FOUR SPECIES OF *Hybognathus* AND OUTGROUPS *Dionda*, *Campostoma*, AND *Pimephales*. The number of individuals of each genotype is provided in parentheses. No activity (u) was observed for mAat-A for *D. episcopa* and *C. anomalum*.

	Hybognathus							*Dionda episcopa*	*Campostoma anomalum*	*Pimephales promelas*
	placitus		*amarus*		*nuchalis*	*hankinsoni*				
	Revuelto Creek	Pecos River	Rio Grande 1	Rio Grande 2	Buffalo River	L. W. canal 1	L. W. canal 2	Pecos River	Arroyo Pecos	Captive
mAat-A	bb (20)	ab (1) bb (18)	bb (20)	bb (7) bc (2)	bb (20)	bb (6)	bb (14) bc (1)	u	u	aa (1) bb (2)
sAat-A	bb (20)	bb (20)	bb (20)	bb (9)	bb (20)	bb (5) bc (1)	bb (14) bc (1)	cc (2) cd (1)	cc (3)	aa (3)
Est-1	aa (15) ab (2) bb (1)	aa (8) ab (7) bb (4) be (1)	cd (1) dd (19)	dd (9)	cc (2) ce (9) ee (8) ef (1)	bb (6)	bb (14)	bb (3)	bb (3)	ab (2) bb (1)
Est-3	bb (19)	bb (16)	cc (19)	cc (9)	bb (18)	aa (6)	aa (14) ad (1)	cc (3)	cc (3)	cc (3)
Est-4	cc (18)	cc (17)	cc (13)	ac (1) cc (8)	cc (18)	cc (6)	cc (15)	bb (3)	bb (3)	bb (3)
G3pdh-A	ac (1) cc (17)	ac (2) cc (18)	cc (20)	bc (1) cc (7)	cc (16) cf (1)	cc (6)	cc (11) cd (1)	cc (2) ff (1)	ee (2) ff (1)	ad (3)
mIcdh-A	aa (20)	aa (19)	aa (19) ab (1)	aa (9)	aa (20)	aa (6)	aa (15)	aa (3)	aa (3)	aa (3)
Ldh-A	aa (20)	aa (20)	aa (20)	aa (9)	aa (20)	aa (6)	aa (15)	dd (3)	dd (3)	bc (1) cc (2)
Ldh-B	aa (20)	aa (20)	aa (20)	aa (8) ab (1)	aa (19) ac (1)	aa (6)	aa (15)	aa (3)	aa (3)	aa (3)
sMdh-A	df (6) ff (14)	df (4) ff (16)	af (2) bf (1) df (2) ff (15)	df (1) ff (8)	ff (20)	ff (6)	ff (15)	ee (3)	ee (3)	cg (3)
sMdh-B	aa (19) ac (1)	aa (19) ac (1)	aa (20)	aa (9)	aa (20)	aa (6)	aa (15)	bb (3)	dd (3)	aa (3)
Pep-1	cc (20)	cc (20)	bc (1) cc (15)	ac(1) cc(8)	cc (20)	cc (6)	cc (15)	cc (3)	cc (3)	aa (3)
Pep-2	cc (1) cd (1) dd (16) de (2)	cd (1) dd (17) de (1) ee (1)	cc (20)	ac (2) cc (7)	aa (7) ab (8) ac (1) bb (4)	aa (5) ac (1)	aa (14) ab (1)	ff (3)	ee (3)	ee (3)
Pep-3	bb (20)	bb (20)	bb (19)	bb (9)	ac (1) bb (19)	bb (6)	bb (15)	bb (3)	bb (2)	bb (3)
Pgdh-A	cc (19) cd (1)	bc (1) be (2) cc (13) cd (1) ce (2) ee (1)	bc (8) bd (1) cc (8) cd (2)	bb (1) bc (3) cc (3)	cc (19) dd (1)	cc (5)	cc (14)	aa (3)	aa (3)	bb (3)
Pgm-A	dd (20)	cd (1) ce (1) dd (16) de (1) df (1)	cc (17) cd (3)	bc (1) cc (2) cd (5) dd (1)	ac (1) bb (1) bc (4) cc (13) cg (1)	dd (6)	dd (15)	ee (3)	cc (3)	ff (3)

TABLE 2. CONTINUED.

	Hybognathus							*Dionda episcopa*	*Campostoma anomalum*	*Pimephales promelas*
	placitus		*amarus*		*nuchalis*	*hankinsoni*				
	Revuelto Creek	Pecos River	Rio Grande 1	Rio Grande 2	Buffalo River	L. W. canal 1	L. W. canal 2	Pecos River	Arroyo Pecos	Captive
Prot-1	aa (20)	aa (20)	aa (20)	aa (9)	aa (20)	aa (6)	aa (15)	aa (3)	aa (3)	aa (3)
Prot-2	bb (19)	bb (13)	bb (16)	ab (1) bb (8)	bb (20)	bb (6)	bb (15)	cc (3)	bb (3)	bb (3)
Sod-1	aa (20)	aa (20)	aa (20)	aa (9)	aa (20)	aa (6)	aa (15)	aa (3)	aa (3)	aa (3)
Sod-2	ad (1) dd (19)	dd (18)	aa (17) ac (1)	aa (8)	bb (18)	dd (4)	ad (3) dd (6)	bb (3)	bb (3)	ad (3)
Sod-3	aa (20)	aa (20)	aa (20)	aa (9)	aa (20)	aa (6)	aa (15)	aa (3)	aa (3)	aa (3)
Mdh-A	aa (20)	aa (20)	aa (18)	aa (9)	aa (20)	aa (6)	aa (15)	bb (3)	aa (1) ac (1) cc (1)	aa (2) ab (1)
Mean number of alleles/ locus	1.4	1.6	1.5	1.5	1.5	1.1	1.3	1.1	1.1	1.3
Percentage of loci polymorphic	13.6	27.3	13.6	45.5	22.7	9.1	4.5	9.1	9.1	31.8
Mean heterozygosity	0.035	0.064	0.054	0.101	0.064	0.015	0.031	0.015	0.015	0.197
SE	0.015	0.024	0.028	0.035	0.032	0.010	0.016	0.015	0.015	0.078

(Rogers, 1972; Nei, 1978). The matrix of Nei's (1978) unbiased genetic distance was summarized by the unweighted pair group method with arithmetic averaging (UPGMA).

Phylogenetic trees were generated using two methods. All Wagner trees were rooted by independently designating *Pimephales*, *Campostoma*, and *Dionda* as outgroups. Distance Wagner trees were produced in BIOSYS-1 from a matrix of Rogers' (1972) distance values following the recommendation of Rogers (1986).

FREQPARS (Swofford and Berlocher, 1987) was also used to generate modified Wagner trees. This procedure determines the tree on which the frequency of each allele undergoes the least possible amount of change, while ensuring that allele frequencies in hypothetical ancestors meet the additivity requirement. Due to the limited tree finding ability of this program, 13 different ingroup tree topologies for the four species of *Hybognathus* were input (USERTREE option). The outgroup topology ([*Dionda* + *Campostoma*] + *Pimephales*), as determined by the Distance Wagner analysis and supported by Mayden (1989), was held constant. Because few individuals (n = 3) were examined for each of the outgroups, FREQPARS was not used to examine outgroup relationships due to the potential for inaccurate estimation of gene frequencies in these taxa. Phylogenetic relationships of *Hybognathus* have been proposed (Schmidt, 1989), and the topology of that cladogram also was tested by eliminating the species of *Hybognathus* not considered in this study.

Genetic similarities among populations were further summarized by principal component analysis (Statistical Analysis Systems [SAS], 1985) of the covariance matrix derived from arcsine transformed allelic frequencies (Gorman and Gaines, 1987). For the 19 polymorphic loci, only the predominant allele was transformed and included in these analyses.

RESULTS

Allozyme variation.—Gene products of 22 presumptive loci were resolved and genotype arrays and measures of genetic variability were calculated (Table 2). Estimates of intrapopulation variability for the species of *Hybognathus* were within ranges reported for populations and species of other cyprinids (Avise, 1977; Buth,

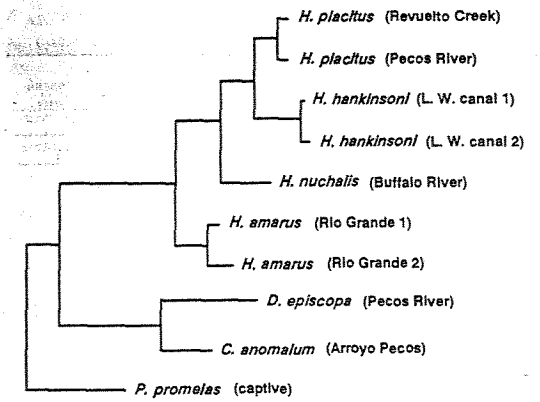

Fig. 1. Distance Wagner tree derived from Rogers' (1972) genetic distance values. All tree lengths are proportional and the cophenetic correlation for this tree was 0.988. The modified Distance Wagner analysis (FREQPARS) calculated a shortest tree that had the same topology.

1984). Mean number of alleles per locus averaged 1.6, and mean percentage of polymorphic loci was 23.2.

Levels of allozyme differentiation were consistent with the current taxonomic recognition of all the species examined, including *H. amarus*. Four fixed electromorphic differences occurred between *H. nuchalis* and *H. hankinsoni* (Sod-2, Est-1, Est-3, Pgm-A) and two fixed electromorphic differences existed between species pairs *H. amarus* and *H. nuchalis* (Sod-2, Est-3), *H. amarus* and *H. placitus* (Est-1, Est-3), and *H. amarus* and *H. hankinsoni* (Est-1, Est-3). A single fixed difference occurred between *H. placitus* and *H. nuchalis* (Sod-2) and between *H. hankinsoni* and *H. placitus* (Est-3). These fixed electromorphic differences between all species comparisons indicate that there was no recent exchange of genes among these forms and support their recognition as distinct species.

Genetic distances (D; Nei, 1978) generated from the allozymic data ranged from 0.001 to 1.001. Genetic distance values were greater than 0.111 for all intrageneric pairwise comparisons of species and greater than 0.275 for intergeneric comparisons. Average genetic distances between intraspecific samples were 0.001 in *H. hankinsoni*, 0.004 in *H. amarus*, and 0.005 between samples of *H. placitus*.

The UPGMA phenogram (cophenetic correlation = 0.977) revealed three major clusters as follows: (1) the four species of *Hybognathus*, (2) *Pimephales*, and (3) *Dionda* and *Campostoma*. Within *Hybognathus*, all intraspecific samples clustered together. *Hybognathus placitus* and *H. hankinsoni*, together with *H. nuchalis* and *H. amarus*, respectively, joined this cluster at increasing distances.

The branching pattern of the ingroup taxa for the Distance Wagner tree (Fig. 1) was identical to the topology of the UPGMA phenogram and remained the same regardless of whether *Dionda*, *Campostoma*, or *Pimephales* were specified as outgroups. The shortest FREQPARS tree (length = 72.0) was also identical in ingroup topology, whereas the next shortest tree (length = 73.4) also placed *H. amarus* as a sister taxon to the other three taxa ([(*H. nuchalis* + *H. hankinsoni*) + *H. placitus*] + *H. amarus*). Analysis of Schmidt's (1989) topology increased the length of the tree (length = 79.2).

The principal component analysis (PCA) reduced the dimensionality of this data set and revealed that the loci separated the species along the first three axes, while maintaining intraspecific population groupings (Fig. 2). The first three factors of the analysis explained 88% of the variation. Factor 1 explained 65% of the variance, and factor loadings were high and positive for all loci except for Ldh-B, mIcdh-A, Pep-3, and Est-1, which had low positive or negative loadings. Factor 1 primarily distinguished the species of *Hybognathus* from the three outgroup genera. Factors 2 and 3 explained 14% and 9% of the variation, respectively, and separated the four species of *Hybognathus*.

We found evidence for past genic introgression between introduced *H. placitus* and native *H. amarus* in the Pecos River. Five of 20 specimens from the Pecos River sample of *H. placitus* had alleles characteristic of *H. amarus*. These alleles were not detected in the sample of 20 specimens of *H. placitus* sampled from the Canadian River (a separate drainage from the Rio Grande). Two *H. placitus* from the Pecos River had the Pgm-A c allele of *H. amarus*, whereas another three individuals had the Pgdh-A b allele of *H. amarus*. The Pgm-A c allele also occurred in *H. nuchalis* and *C. anomalum*, whereas the Pgdh-A b allele was found only in *H. amarus* and *P. promelas*.

Discussion

Electrophoretic investigations of cyprinids have revealed substantial heterogeneity in the level of genetic divergence at the species level (reviewed by Buth, 1984; Dowling and Brown, 1989). Inclusion of two samples in this study of *H. amarus*, *H. placitus*, and *H. hankinsoni* pro-

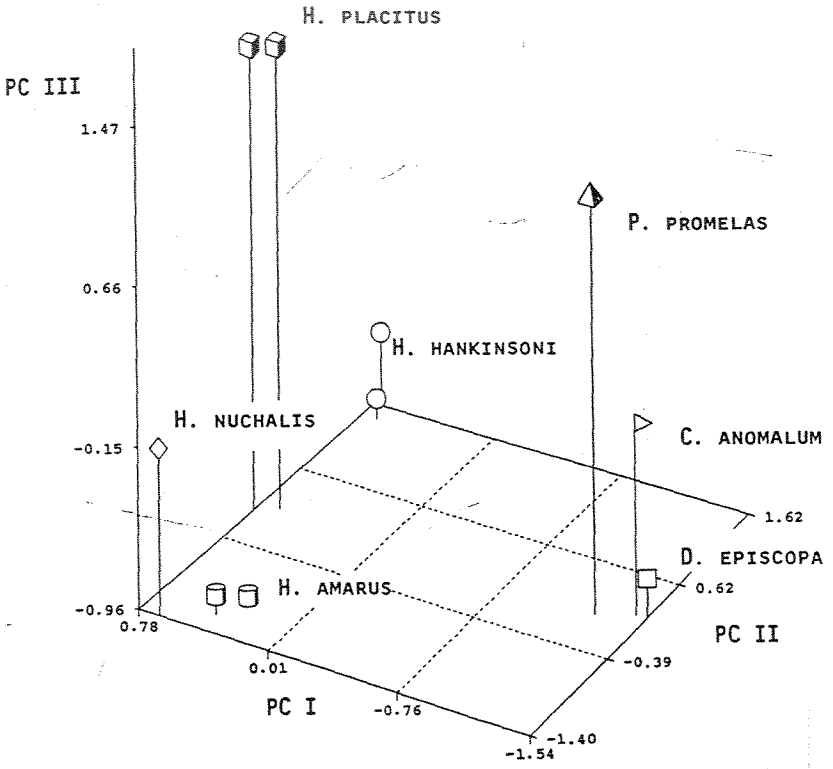

Fig. 2. Principle components analysis of the arcsine transformed dominant allele frequency revealed three factors were important in discriminating among the groups. Factor 1 explained 65% of the variation; Factor 2 explained 14%; and Factor 3 explained 9%.

vided an estimate of the level of intraspecific variation in this genus. Genetic distance values are greater than 0.111 for all the pairwise species comparisons in this study, whereas intraspecific genetic distances are less than 0.005. These values are within the range of inter- and intraspecific values, respectively (often reported as Nei's *I* value), reported in other cyprinid allozyme studies (summarized by Buth, 1984).

Principle component analysis of allozymic data has been shown to effectively summarize genetic variation among groups (Gorman and Kines, 1987; Rainboth et al., 1989), and PCA supported specific distinction of the four species of *Hybognathus* we examined. Intraspecific samples were tightly grouped, whereas species were clearly distinguished by Factors 2 and 3. Thus, there is little justification, as judged by phenetic evidence, for considering the Rio Grande form of *Hybognathus, H. amarus,* conspecific with either *H. nuchalis* or *H. placitus,* species with which it previously has been in synonymy.

This observation is further supported by the phylogenetic analyses. In none of the shortest trees was *H. amarus* depicted in a sister-group relationship with *H. placitus* or *H. nuchalis.* The Distance Wagner and FREQPARS analyses placed *H. hankinsoni* and *H. placitus* together in a clade. *Hybognathus nuchalis* was a sister taxon to this clade, and *H. amarus* was a sister species to the clade including those three *Hybognathus.*

Without a phylogenetic analysis, Hlohowskyj et al. (1989) suggested that *H. amarus* and *H. placitus* were sister taxa based on shared similarities in the pharyngeal filtering apparatus. However, our results indicate that these similarities represent convergence or retention of a primitive character. Furthermore, none of our trees supported the suggestion by Hlohowskyj et al. (1989) that *H. hankinsoni* may be primitive for the genus.

Our results also contrast with those that Schmidt (1989) obtained in an osteological analysis of phylogenetic relationships within the genus *Hybognathus.* He concluded that there is an unresolved trichotomy among *H. regius, H. hankinsoni,* and a clade comprised of *H. placitus, H. hayi, H. nuchalis, H. argyritis,* and *H. amarus.* He

resolved the latter clade into one containing *H. placitus* and one containing the remaining four species. Within this clade, *H. hayi* was divergent, and *H. amarus, H. argyritis,* and *H. nuchalis* were indistiguishable. Our allozymic study does not include all nominal forms of *Hybognathus* addressed by Schmidt (1989); however, our allozyme data do not support Schmidt's hypothesized relationships for the four species of *Hybognathus* included in this study.

Relationships at the generic level also may be addressed by these data, although the sample sizes were small. Whether analyzed cladistically or phenetically, the allozymic data clustered *Dionda* and *Campostoma* separate from *Pimephales,* a result also noted by Cavender and Coburn (1988) and Schmidt (1989). The relationships of these genera to *Hybognathus* are less clear, however. The UPGMA analysis suggested that *Pimephales* is more similar to *Hybognathus* than are *Dionda* and *Campostoma.* This is contrary to the morphologic analyses of Cavender and Coburn (1988), Mayden (1989), and Schmidt (1989). The shortest Distance Wagner tree, derived when *Pimephales* was designated the outgroup, included *Campostoma* and *Dionda* as a sister group to the clade comprised of the species of *Hybognathus.* The relationships of these genera should be further examined with larger sample sizes and more species.

Although the data suggesting genic introgression between *H. amarus* and *H. placitus* are equivocal, due to the possibility that these characters are convergent or primitively shared, the hybridization hypothesis is supported by morphological evidence from putative hybrids of *H. amarus* × *H. placitus* collected from the Pecos River in 1964 (ASU 1308). In these specimens, the basioccipital process width is intermediate in shape and size between the long, narrow process of *H. placitus* and the short, wide process of *H. amarus* (unpubl. data). Based upon museum material and our recent collections, *H. placitus* was introduced into the Pecos River, New Mexico, prior to 1964 and since that time has completely replaced *H. amarus.*

The Rio Grande basin is a center of cyprinid endemism; yet few phylogenetic studies have included members of this fauna, precluding a thorough understanding of the zoogeography of the region (Smith and Miller, 1986). Due to species extirpations and the rapid change in faunal composition of many of the drainages in the Rio Grande basin in the past few decades, the necessity of elucidating zoogeographic and systematic relationships is evident. We will never known the extent of genetic variation in *H. amarus,* a species that once occurred in the Pecos River and as far downstream in the Rio Grande as Brownsville, Texas, but that is now limited to a short reach of the Rio Grande in central New Mexico (Bestgen and Platania, 1991).

The analyses provided in this paper support recognition of the Rio Grande silvery minnow, *H. amarus,* as a valid taxon. However, its validation as a species may be little more than an epitaph if efforts to conserve it are not soon forthcoming. Since the 1930s, five forms have been extirpated from the Rio Grande in New Mexico and two (*Notropis orca* and *N. simus simus*) are extinct (Chernoff et al., 1982; Propst et al., 1987; Bestgen and Platania, 1990). The loss of these species has occurred primarily because of human modification (physical and biological) of the river system, modifications that may be diminishing the range of the once more widely distributed *H. amarus.*

Material Examined

Sample localities (University of New Mexico, Museum of Southwestern Biology collection number) and [number of specimens examined electrophoretically] of the 119 individuals included in this study are listed below.

Hybognathus placitus.—New Mexico: Quay County, Revuelto Creek W of Logan at State 38 crossing, T13N R33E S24 (MSB 4634) [n = 20]; Chaves County, Pecos River at Lake Arthur Falls, T15S R26E S26 (MSB 4646) [n = 20].

Hybognathus amarus.—New Mexico: Socorro County, Rio Grande, 2 km N of US 380 crossing, T4S R1E S21 (MSB 4636) [n = 20]; Sandoval County, Rio Grande, Bernalillo Bridge to 4 km S (MSB uncatalogued) [n = 9].

Hybognathus nuchalis.—Mississippi: Wilkinson County, Buffalo River, 21 km SW Hwy 61 on Sanders Fork Creek Bridge (MSB 4807) [n = 20].

Hybognathus hankinsoni.—Colorado: Larimer County, Larimer Weld canal at Hwy 14, east of Fort Collins (MSB 4647) [n = 6]; (MSB 4806) [n = 15].

Dionda episcopa.—New Mexico: Eddy County, 27 km W of Carlsbad, Rocky Arroyo, T21S R24E S28 (MSB 4648) [n = 3].

Campostoma anomalum.—New Mexico: San Miguel County, Arroyo Pecos, 1 km N of Las Vegas (MSB uncatalogued) [n = 3].

Pimephales promelas.—New Mexico: Bernalillo County, Albuquerque, Quality Bait Shop (captive; MSB uncatalogued) [n = 3].

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