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Known Range of the Manantial Roundnose Minnow, *Dionda
argentosa***

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CONSERVATION GENETICS OF CYPRINID FISHES (GENUS *DIONDA*) IN SOUTHWESTERN NORTH AMERICA. II. EXPANSION OF THE KNOWN RANGE OF THE MANANTIAL ROUNDNOSE MINNOW, *DIONDA ARGENTOSA*

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ABSTRACT—Cyprinids belonging to the genus *Dionda* have a relatively broad distribution in southwestern North America. However, validity of populations in several of the nominal species has not been examined rigorously. During the course of a conservation-genetics study of *Dionda* in western Texas we determined that one population presumed to represent *Dionda episcopa* instead belongs to *Dionda argentosa*. This extends the native range of *D. argentosa* to include the lower Pecos River basin in addition to its currently recognized distribution in three tributaries of the Rio Grande. Conservation implications for both species are discussed.

RESUMEN—Ciprínidos pertenecientes al género *Dionda* tienen una distribución relativamente amplia en el suroeste de Norte América. A pesar de esto, la validez de poblaciones en varias especies nominales no ha sido examinada rigurosamente. Durante un estudio genético para la conservación de *Dionda* en el oeste de Texas, determinamos que una población que se presumía pertenecer a la especie *Dionda episcopa*, pertenece en cambio a *Dionda argentosa*. Este hallazgo extiende la distribución nativa de *D. argentosa* para incluir la cuenca baja del río Pecos además de su distribución reconocida actual en tres tributarios del río Grande. Adicionalmente, discutimos implicaciones para la conservación de las dos especies.

Cyprinids of the genus *Dionda* are common components of headwater spring and stream systems in southwestern North America (Mayden et al., 1992; Miller, 2005). However, native ranges of most species of *Dionda* are fairly restricted, and their conservation status in the United States ranges from unlisted in New Mexico and from special concern (at risk of decline) to state and federal listings as threatened in Texas (United States Fish and Wildlife Service, 1999; Texas Wildlife Action Plan, http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_pl_w7000_1187a/). The Manantial roundnose minnow (*Dionda argentosa*) is one of the species of *Dionda* considered to be of special concern (Texas Wildlife Action Plan, http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_pl_w7000_1187a/) and is believed to be endemic to the Devils River, San Felipe Creek, and Sycamore Creek, three tributaries of the Rio Grande in western Texas (Hubbs et al., 1991). The roundnose minnow, *Dionda episcopa*, presently is considered to be of special concern in Texas

(Texas Wildlife Action Plan, http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_pl_w7000_1187a/) and was listed as threatened in New Mexico until 1983, after which it was removed from the list (Sublette et al., 1990). *Dionda episcopa* is believed to occur over a broader range that includes the upper and lower portions of the Pecos River in Texas and New Mexico, as well as the Big Bend region of the Rio Grande (Sublette et al., 1990; Scharpf, 2005). Assignment of some populations of *Dionda* to a given species, however, reflects historical considerations of distributions of individual species rather than detailed study and comparison to well-studied exemplars. In this note, we report that one population of *Dionda* previously considered to be *D. episcopa* is, instead, *D. argentosa*.

Our report is part of an ongoing population-genetics survey of the five species of *Dionda* that occur in Texas and was based on a sample of 223 individuals obtained from nine localities in Texas and one in New Mexico (Table 1). Specimens were assigned initially to species

TABLE 1—Collection localities of *Dionda* assayed for sequences of the mitochondrially encoded ND-5 gene. Species assignments indicate current perceptions as to species within various drainages.

Species	Locality	Drainage	Latitude	Longitude	<i>n</i>	Number of haplotypes
<i>Dionda argentosa</i>	Devils River, Val Verde County, Texas	Rio Grande	29°53'20.84"N	100°59'18.35"W	26	7
	San Felipe Creek, Val Verde County, Texas	Rio Grande	29°21'52.38"N	100°53'9.50"W	20	3
<i>Dionda diaboli</i>	Devils River, Val Verde County, Texas	Rio Grande	29°53'37.95"N	100°59'43.65"W	23	7
	Pinto Creek, Kinney County, Texas	Rio Grande	29°24'39.09"N	100°27'5.29"W	21	3
<i>Dionda episcopa</i>	El Rito Creek, Chaves County, New Mexico	Upper Pecos River	33°18'17.10"N	104°41'0.95"W	22	1
	Independence Creek, Terrell County, Texas ^a	Lower Pecos River	30°28'4.63"N	101°48'8.21"W	26	3
<i>Dionda nigrotaeniata</i>	Comal Springs, Comal County, Texas	Guadalupe River	29°43'5.27"N	98°7'53.36"W	20	1
	Fessenden Spring, Kerr County, Texas	Guadalupe River	30°10'0.51"N	99°20'36.60"W	20	2
<i>Dionda serena</i>	Frio River, Uvalde County, Texas	Nueces River	29°50'45.30"N	99°46'18.23"W	21	5
	Nueces River, Uvalde County, Texas	Nueces River	29°48'42.24"N	100°0'56.45"W	24	14

^a Phylogenetic analysis of mitochondrial genes ND-5 and cytochrome-*b* indicate that this population is *Dionda argentosa*, which has been corroborated with morphological evaluation.

based on available information (Hubbs et al., 1991; Garrett et al., 2004; Bonner et al., 2005) as to species of *Dionda* within various drainages. The sample of *Dionda* from Independence Creek was, thus, initially designated as *D. episcopa* because of its location in the Pecos River drainage (following Bonner et al., 2005). All specimens were collected by seine and each specimen was preserved whole in 95% ethanol. Specimens from Texas were obtained under permits SPR-0390-045 from the Texas Parks and Wildlife Department and TE676811 from the United States Fish and Wildlife Service; specimens from New Mexico were kindly provided by T. Krabbenhoft (permit 1896 from the New Mexico Department of Game and Fish).

DNA was extracted from clips of fins or from muscle tissue, using the protocol of Sambrook et al. (1989). Polymerase chain reaction (PCR) amplification of a 597 base-pair (bp) fragment of the mitochondrial protein-coding NADH dehydrogenase subunit 5 gene (ND-5) employed primers L12328 (5'-AACTCTTGGTGCAAMTC-CAAG-3'; Miya et al., 2006) and DS-H (5'-AAAAATTTGTTGAATTTCTCAGGA-3'; E. W.

Carson and A. H. Hanna, unpublished data). Amplification conditions were 95°C for 3 min, 35 cycles of 95°C for 30 s, 48°C for 1 min, 72°C for 1 min, followed by a 10-min extension at 72°C. Total PCR products for each amplification were electrophoresed on a 2% agarose gel and target fragments obtained via band cutting. Fragments were cleaned with a QIAquick Gel Extraction Kit (Qiagen, Valencia, California), following directions of the manufacturer. Sequencing reactions employed the L12328 (forward) primer and Big Dye terminators (Applied Biosystems, Foster City, California); DNA sequencing was done on an ABI 3100 (Applied Biosystems, Foster City, California). Sequences were aligned and protein coding verified in SEQUENCHER 4.1 (Gene Codes, Ann Arbor, Michigan). Errors or ambiguities were corrected after visual inspection of chromatograms or through re-sequencing problematic samples. Due to inconsistent sequencing of the terminal 12 bp of the ND-5 fragment, mtDNA sequences used in subsequent analysis were truncated to 585 orthologous bases. A total of 41 mtDNA haplotypes (Table 1) was identified across all individuals assayed; individual

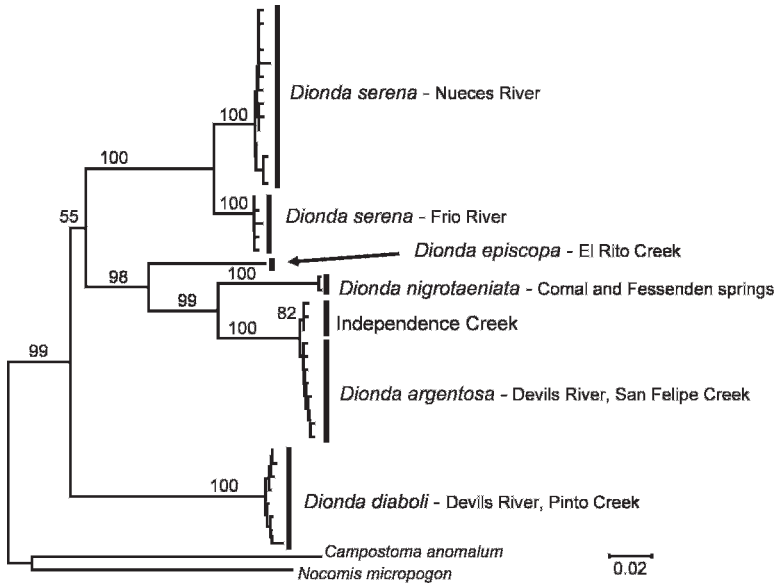


FIG. 1.—Neighbor-joining tree based on ND-5 haplotypes. Levels of bootstrap support are indicated by numbers on branches. A scale of genetic distance is at the bottom of the figure.

haplotypes, by sampling locality and species, are in GenBank (Accessions GU252301–GU252341) and at <http://wfsc.tamu.edu/doc/> under the file name mtDNA sequences of *Dionda*.

Neighbor-joining and maximum-parsimony analyses of ND-5 sequences were implemented in MEGA, version 4.0 (Tamura et al., 2007; www.megasoftware.net/). The Jukes-Cantor method was applied for neighbor joining, whereas maximum parsimony employed the heuristic search option, with 10 random-addition replicates. Maximum-likelihood analysis employed RAxML 7.0.4 (Stamatakis et al., 2005, 2008; CIPRES Cluster, San Diego Supercomputing Center), with the best evolutionary model (GTR + G) determined in jModelTest version 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008). Each phylogenetic analysis included all 41 observed mtDNA haplotypes. Orthologous mtDNA sequences from *Campostoma anomalum* (one individual; GenBank Accession GU252342) and *Nocomis micropogon* (one individual; GenBank Accession GU252343), two genera closely related to *Dionda* (Mayden et al., 1992; Schönhuth et al., 2008), were used as outgroups. A total of 1,000 bootstrap pseudoreplicates was used to assess robustness of inferred relationships in neighbor-joining and maximum-parsimony analyses;

the number of bootstrap pseudoreplicates in maximum-likelihood analysis was determined automatically by RAxML. Strong bootstrap support (100%) in the neighbor-joining tree (Fig. 1) was shown for a lineage that included haplotypes from Independence Creek (identified initially as a location for *D. episcopa*) and haplotypes from the Devils River and San Felipe Creek (type localities of *D. argentosa*; Girard, 1856). In addition, there was strong bootstrap support (99%) for a sister-group relationship between samples of *D. argentosa* (including the sample from Independence Creek) and *D. nigrotaeniata*, with the haplotypes of *D. episcopa* from El Rito Creek forming a sister group (98% bootstrap support) to the clade comprised of *D. argentosa* and *D. nigrotaeniata* (Fig. 1). Virtually identical results were revealed in both maximum-parsimony and maximum-likelihood topologies (available from EWC).

To place these findings within the context of the mitochondrial cytochrome-*b*-based phylogeny of *Dionda* published recently by Schönhuth et al. (2008), we sequenced a 1,140 bp fragment of the cytochrome-*b* gene from a single individual from Independence Creek (GenBank Accession GU252344). We used PCR primers LA and HA and experimental protocols of Schmidt et al.

(1998) for PCR amplification and sequencing, and used orthologous sequences from the GenBank submissions (Accessions: EU082498–EU082499 for *D. argentosa*; DQ324085–DQ324086 and EU082493 for *D. diaboli*; DQ324077–DQ324079 and EU082490 for *D. episcopa*; EU082501–EU082503 for *D. nigrotaeniata*; and DQ324080 and EU082504 for *D. serena*) of Schönhuth et al. (2008) to assess phylogenetic placement of the cytochrome-*b* haplotype from Independence Creek relative to cytochrome-*b* haplotypes of the five species of *Dionda* (*D. argentosa*, *D. diaboli*, *D. episcopa*, *D. nigrotaeniata*, and *D. serena*) that occur in Texas. Orthologous cytochrome-*b* sequences from *C. anomalum* and *N. leptocephalus* (GenBank Accessions DQ324063 and EU082468, respectively) were used as outgroups. Neighbor-joining, maximum-likelihood, and maximum-parsimony methods again were used to assess phylogenetic relationships. Parameters for each method of analysis were as described above, except that the GTR + G + I evolutionary model (based on results from jModelTest) was used for maximum-likelihood analysis. Strong bootstrap support (99–100%) was observed in all three phylogenetic approaches (topologies available from EWC) for (i) a clade that included the haplotype of *D. argentosa* from San Felipe Creek, the haplotype from Pandale, Val Verde County, Texas (designated as *D. argentosa* by Schönhuth et al., 2008), and the haplotype from Independence Creek, and (ii) a sister-group relationship between *D. argentosa* and *D. nigrotaeniata*, with haplotypes of *D. episcopa* forming a clade that was sister to the *D. argentosa*-*D. nigrotaeniata* clade.

Specimens of *Dionda* collected from Independence Creek keyed morphologically to *D. argentosa*, using the taxonomic key of Hubbs et al. (1991). Museum collections of *Dionda* at the Texas Cooperative Wildlife Collection (TCWC) at Texas A&M University were then examined, as were online records (<http://www.utexas.edu/tmm/tnhc/fish/>) at the Texas Natural History Collections (TNHC) at the University of Texas at Austin, to assess how *Dionda* from prior collections in the Pecos River and other drainages in Texas had been classified. Most collections of *Dionda* from the Pecos River drainage (both museums) were identified as *D. episcopa*. However, one collection at the TCWC, sampled from Independence Creek in 1994, was identified as *D. argentosa*, as was one collection from Independence Creek housed at the TNHC. Neither

of these identifications made their way into the published literature. Complicating matters, several other collections of *Dionda* from Independence Creek (housed at the TNHC) were identified as *D. episcopa*. Perhaps more telling, is that numerous collections of *Dionda* (both museums) from various localities in Texas remain identified as *D. episcopa*, although they were collected at localities now known to represent other species of *Dionda* (e.g., *D. nigrotaeniata* and *D. serena*). This includes collections from near Pandale, Texas, that remain identified as *D. episcopa*, although they almost certainly are *D. argentosa*. These observations underscore the problems with taxonomy of *Dionda* and the need for proper documentation of which species of *Dionda* occur where.

Our study demonstrates that *Dionda* in Independence Creek are not *D. episcopa*, but are a previously unrecognized population of *D. argentosa*. This appears not to be an artifact of historical introgressive hybridization, as initial results from microsatellite genotyping (data not shown) indicate that *Dionda* from Independence Creek align with *D. argentosa* from the Devils River and San Felipe Creek and not with *D. episcopa* from El Rito Creek. It is not necessarily surprising that *D. argentosa* occurs in the lower Pecos River basin as well as the three tributaries of the Rio Grande, as two other freshwater fishes, *Cyprinella proserpina* and *Etheostoma grahami*, also are endemic to these same general localities (Hubbs et al., 1991). In addition, Schönhuth et al. (2008) reported that mitochondrial cytochrome-*b* and nuclear Rag1 and S7 sequences of a single individual sampled from the lower Pecos River at Pandale grouped closely with homologous sequences of *D. argentosa* sampled from San Felipe Creek. As Independence Creek is ca. 45 river-km northwest (upstream) of the Pecos River at Pandale, it is possible that *D. argentosa* has a broad distribution within this drainage.

A corollary to range expansion of *D. argentosa* in the Pecos River is a possible reduction in the known range of *D. episcopa*. Presently, *D. episcopa* is believed to occur in the upper and lower portions of the Pecos River, as well as the Big Bend region of the Rio Grande (Sublette et al., 1990; Scharpf, 2005). Schönhuth et al. (2008) sequenced the cytochrome-*b* gene from individuals sampled at five localities in the Pecos River basin (El Rito Creek, Bitter Lakes, Black

River Village, Limpia Creek at Fort Davis, and Pandale). The haplotype sampled at Pandale grouped with *D. argentosa*, whereas haplotypes from the other four localities formed a monophyletic clade. These results suggest that *D. episcopa* could be limited in Texas to the Pecos River upstream of the confluence with Independence Creek, although the precise limit of range downstream remains unknown. Given that *D. episcopa* is considered of special concern in Texas, a more thorough survey of *Dionda* in the lower Pecos River and in the Rio Grande could yield further range reduction of *D. episcopa* and, perhaps, a change in its conservation status in Texas. Conversely, the geographic distribution of *D. argentosa* is broader than currently appreciated, meaning that its status as special concern in Texas might need to be re-evaluated. A thorough, genetics-based survey of populations of *Dionda* throughout the region is warranted, as effective conservation of individual species requires accurate knowledge of their distributions.

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