Phylogeny, diversity, and species delimitation of the North American Round-Nosed Minnows (Teleostei: *Dionda*), as inferred from mitochondrial and nuclear DNA sequences

Susana Schönhuth [a,*], David M. Hillis [b], David A. Neely [c], Lourdes Lozano-Vilano [d], Anabel Perdices [e], Richard L. Mayden [a]

[a] Department of Biology, Saint Louis University, 3507 Laclede Avenue, St. Louis, MO 63103, USA
[b] Section of Integrative Biology, and Center for Computational Biology and Bioinformatics, University of Texas, One University Station C0930, Austin, TX 78712-0253, USA
[c] Tennessee Aquarium Research Institute, Cohutta, GA 30710-7504, USA
[d] Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Nuevo León, Mexico
[e] Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain

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ABSTRACT

Accurate delimitation of species is a critical first step in protecting biodiversity. Detection of distinct species is especially important for groups of organisms that inhabit sensitive environments subject to recent degradation, such as creeks, springs, and rivers in arid or semi-desert regions. The genus *Dionda* currently includes six recognized and described species of minnows that live in clear springs and spring-fed creeks of Texas, New Mexico (USA), and northern Mexico, but the boundaries, delimitation, and characterization of species in this genus have not been examined rigorously. The habitats of some of the species in this genus are rapidly deteriorating, and many local populations of *Dionda* have been extirpated. Considering the increasing concerns over degradation of their habitat, and pending a more detailed morphological revision of the genus, we undertook a molecular survey based on four DNA regions to examine variation over the range of the genus, test species boundaries, and infer phylogenetic relationships within *Dionda*. Based on analyses of two mitochondrial (*cytb* and *D-loop*) and two nuclear (*Rag1* and *S7*) DNA regions from specimens collected throughout the range of *Dionda*, we identified 12 distinct species in the genus. Formerly synonymized names are available for two of these species, and four other species remain undescribed. We also redefine the known range of six species. The limited distribution of several of the species, coupled with widespread habitat degradation, suggests that many of the species in this genus should be targets for conservation and recovery efforts.

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1. Introduction

Accurate and rapid delimitation and identification of species, evolutionarily significant units (ESUs), or designatable units (DUs) is a critical first step in protecting biodiversity in conservation efforts. Unfortunately, arguments over different approaches or species concepts can impede our understanding of biodiversity (Rojas, 1992; Waples, 1998; Agapow et al., 2004; George and Mayden, 2005; Waples et al., 2007). Traditional methods for detecting and describing species are often slow, so that distinctive units that are appropriate targets for conservation often go undetected for long periods of time. In areas experiencing rapid habitat degradation or loss, molecular analyses can help to speed this process of species discovery and description—the critical first steps in the preservation of biodiversity.

Loss of biodiversity from habitat degradation is especially acute in spring-fed freshwater systems in arid and semi-arid regions. The North American Round-Nosed Minnows (genus *Dionda*) inhabit springs and spring-fed creeks and rivers of southwestern Texas, southern New Mexico (USA), and northern Mexico (MX). These freshwater systems are subject to rapid and severe exploitation and habitat degradation by humans.

The genus *Dionda* is characterized by a prominent dark lateral stripe running from the tip of the snout to the caudal base and ending in a black basicaudal spot; a round nose; a subterminal mouth; and the absence of maxillary barbels (Girard, 1856). Species comprising this genus have a controversial taxonomic history, and recognition of the nominal species has changed over the years (see Table 1 for taxonomic background). For many years, *Dionda episcopa* was considered a single polytypic species ranging from the

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* Corresponding author. Fax: +1 314 977 3858.
E-mail address: susana.schonhuth@gmail.com (S. Schönhuth).

Table 1
Original descriptions, previous taxonomies, and recommended current taxonomy for species of the genus *Dionda* (*sensu stricto*).

Original description/type locality Quotes for all the type localities from the original papers, clarifying remarks in brackets	Taxonomy after Jordan and Gilbert (1883), Jordan (1885), Meek (1904), and Miller (1991)	Taxonomy after Mayden et al. (1992) and Gilbert (1998)	Recommended taxonomy (this paper)	Known distribution
Dionda argentosa Girard 1856 "San Felipe Creek and Devil's River, Rio Grande del Norte". [Two tributaries of lower Rio Grande, Val Verde Co., Texas, USA]	*D. episcopa*	*D. argentosa*	*D. argentosa* Girard 1856	Lower Pecos River, Devils River, and nearby tributaries of the middle Rio Grande in Texas, USA and Coahuila, MX
Dionda diaboli Hubbs and Brown 1956 "Devils River at Baker's Crossing, Val Verde Co., Texas, USA"	*D.diaboli*	*D. diaboli*	*D. diaboli* Hubbs and Brown 1956	Devils River and nearby tributaries of the middle Rio Grande, Texas, USA, and Coahuila, MX
Dionda episcopa Girard 1856 "Head waters of the Rio Pecos, and in Camanche [=Comanche] Spring". [Middle Pecos River, in New Mexico (probably in Eddy Co.), and Texas (Pecos Co.), Rio Grande Dr., Texas, USA]	*D. episcopa*	*D. episcopa*	*D. episcopa* Girard 1856	Springs and tributaries of the middle Pecos River system in Texas and New Mexico, USA
Dionda melanops Girard 1856 "Buena Vista [now called La Jolla del Refugio, 8 km SE Saltillo], Coahuila". [Rio San Juan, MX]	*D. episcopa melanops*	*D. melanops*	*D. melanops* Girard 1856	Rio San Juan and Rio Salado in Coahuila and Nuevo Leon, MX
Dionda papalis Girard 1856 "Delaware Creek, tributary of the Rio Pecos". [Delaware River, tributary of the Pecos River, Culberson Co., Texas, USA]	*D. episcopa*	*D. episcopa*	Junior synonym of *D. episcopa* Girard 1856	See distribution of *D. episcopa*
Dionda serena Girard 1856 "Rio Sabinal, Texas". [Rio Sabinal at Sabinal, Frio River, Uvalde Co., Texas, USA]	*D. episcopa*	*D. serena*	*D. serena* Girard 1856	Upper Frio River system, Texas, USA
Dionda texensis Girard 1856 "Rio Nueces, Texas". [Nueces River, ca. 7 mi. west of Uvalde, Uvalde Co., Texas, USA]	*D. episcopa*	*D. serena*	*D. texensis* Girard 1856	Upper Nueces River system, Texas, USA
Hybognathus flavipinnis (Cope 1880) "Johnson's Fork of the Llano in Kimble County" [Llano River, Kimble Co., Colorado Drainage, Texas, USA]	*D. episcopa*	*D. nigrotaeniata* (based on incorrect interpretation of type locality by Gilbert (1998))	*D. flavipinnis* (Cope 1880)	Guadalupe Dr. and Southern Colorado Dr., Texas, USA
Hybognathus nigrotaeniata (Cope 1880) "Upper waters of Wallace Creek, one of the heads of the Medina". [Headwaters of the Medina River, Bandera Co., San Antonio Dr., Texas, USA]	*D. serena* or *D. episcopa*	Not considered; assumed incorrect type locality for *D. nigrotaeniata*	*D. nigrotaeniata* (Cope 1880)	Upper Medina River system, Texas, USA
Hybognathus punctifer Garman 1881 "Parras and spring near Saltillo, Coahuila". [Rio San Juan, MX].	*D. episcopa melanops*	*D. melanops*	Junior synonym of *D. melanops* Girard 1856	See distribution of *D. melanops*
Undescribed species	*D. episcopa*	*D. sp.* [Rio Conchos]	*D. sp.* 1 [Conchos]	Rio Conchos Dr. in Chihuahua and Durango, MX; and Cibolo Creek in Texas, USA
Undescribed species	Species not yet discovered	*D. sp.* [El Vergel Spring], and *D. sp.* [Ojo de Agua]	*D. sp.* 2 [Tunal]	El Vergel Spring, Zacatecas, MX; Ojo de Agua de San Juan, Durango, MX; endorheic river on Durango – Zacatecas border, MX
Undescribed species	*D. episcopa*	*D. nigrotaeniata* (based on incorrect interpretation of type locality by Gilbert (1998)	*D. sp.* 3 [Colorado]	San Saba and Concho Rivers, Northern Colorado Dr., Texas, USA
Undescribed species	*D. episcopa*	*D. episcopa*	*D. sp.* 4 [upper Pecos]	Upper Pecos River at Santa Rosa, New Mexico, USA

Colorado River in Texas, west into the Rio Grande System, and south to the Rio Conchos, and most of the previously described species over its distribution were considered synonyms (an exception was the sympatric *D. diaboli*). Based on morphological variation, however, some authors argued that "*D. episcopa*" was actually a complex of species (e.g., Hubbs and Brown, 1956). Phylogenetic relationships among species of the traditional genus *Dionda* were first examined in comprehensive allozyme analyses using 32 gene loci (Mayden et al., 1992). These authors resolved the *D. episcopa* complex (including *D. diaboli*) as a monophyletic group. However, within the previously recognized *D. episcopa*, not all populations formed a monophyletic group, and they identified several monophyletic groups referable to nine distinct and diagnosable species within the complex. This allozyme study suggested that *D. episcopa* was restricted to the Rio Grande and tributaries upstream of the Devils River (Mayden et al., 1992). These authors recognized populations formerly called *D. episcopa* in the Devils River and San Felipe Spring as *D. argentosa*; populations from the Nueces and Frio rivers as *D. serena*; and populations in the Colorado and Guadalupe river drainages as an undescribed species. Gilbert (1998) argued that the names *D. nigrotaeniata* and *D. flavipinnis* were both available for the species in the Colorado and Guadalupe drainages, and (using the principle of first reviser) selected the name *D. nigrotaeniata* for this species. Among the Mexican populations, Mayden et al. (1992) recognized *D. melanops* from the Rio San Juan (lower Rio Grande, Nuevo Leon, MX), and suggested that this species also extended into the Rio Salado (lower Rio Grande, Coahuila, MX). These authors also recognized three undescribed species from Mexico: one in the upper Rio Conchos

System (*D. sp.* [Rio *Conchos*]), and two in disjunct, isolated springs of the upper Rio Tunal (Mezquital Drainage) in Durango and Zacatecas, MX (*D. sp.* [Ojo de Agua] and *D. sp.* [El Vergel Spring], respectively) (Fig. 1A). Finally, *Dionda diaboli* is found in several tributaries of the middle Rio Grande in Texas, USA and Coahuila, MX, where it is sympatric with *D. argentosa* and *D. melanops*.

Recently, phylogenetic analyses of southern North American cyprinids (based on mitochondrial and nuclear genes) showed that the traditional genus *Dionda* is not monophyletic (Schönhuth et al., 2006, 2008). Schönhuth et al. (2008) separated a series of species formerly considered part of *Dionda* as a monophyletic group that is not related to the *D. episcopa* complex and described a new genus, *Tampichthys*, with many diagnostic morphological and molecular characters. *Tampichthys* contains six species (*T. catostomops*, *T. rasconis*, *T. dichromus*, *T. erimyzonops*, *T. mandibularis* and *T. ipni*) that inhabit rivers of the Tampico Embayment drainage in northeastern Mexico. Schönhuth et al. (2008) restricted *Dionda* to a northern group of species (*D. episcopa*, *D. argentosa*, *D. diaboli*, *D. melanops*, *D. nigrotaeniata*, *D. serena*) and three undescribed forms (*D. sp.* [Rio Conchos], *D. sp.* [Ojo de Agua] and *D. sp.* [El Vergel Spring]).

Currently, six described species are recognized within the *D. episcopa* complex (*D. episcopa*, *D. serena*, *D. melanops*, *D. argentosa*, *D. nigrotaeniata*, and *D. diaboli*), plus three undescribed species in Mexico (most in drainages of the Gulf of Mexico from the Colorado River to Rio Grande System). From east to west, drainages that contain members of the *Dionda episcopa* complex are the Colorado, Guadalupe, San Antonio, Nueces, Frio, and Rio Grande systems on the Atlantic versant, and the upper Rio
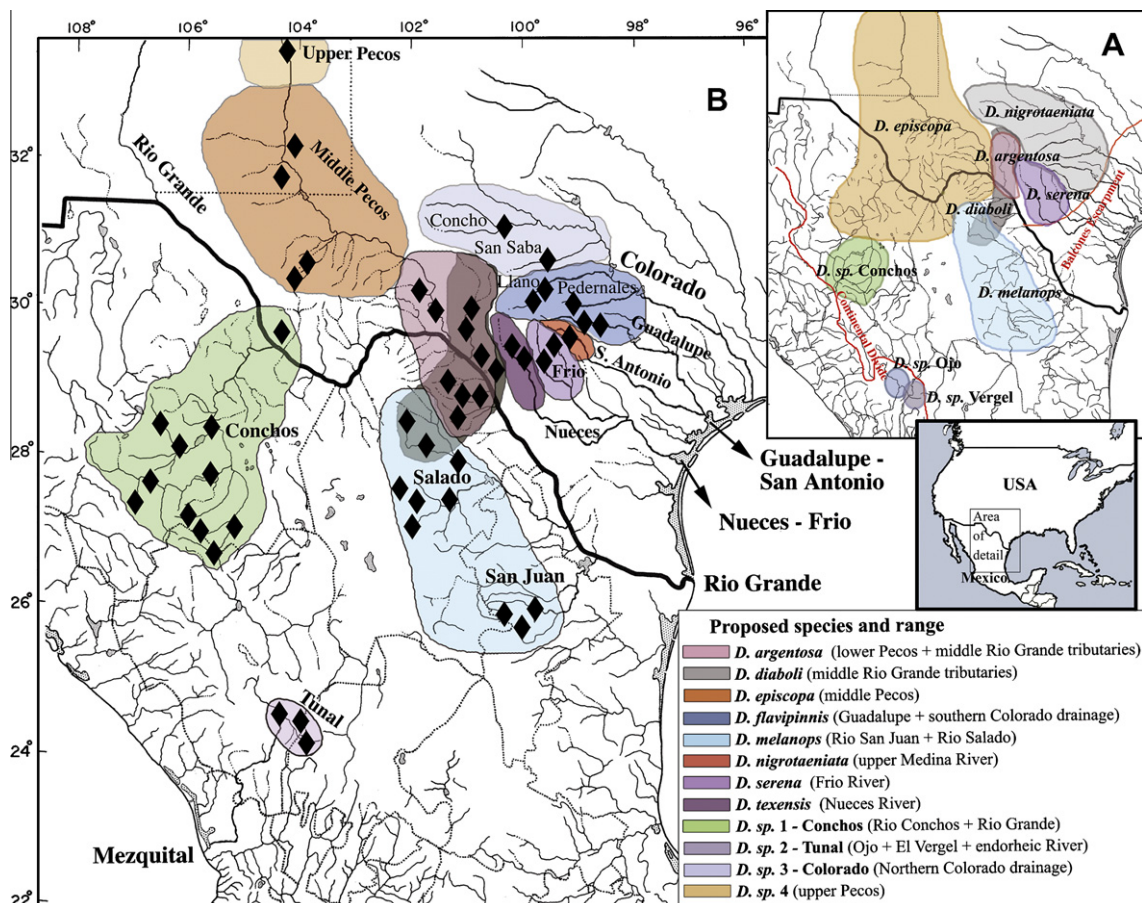
Fig. 1. Distribution of the genus *Dionda* (*sensu stricto*). (A) Currently recognized species. (B) The 12 species recognized in this study, and their ranges, with sampled localities indicated. Adjacent sampling localities in close proximity are combined and shown as a single locality.

Mezquital drainage on the Pacific versant. Species of *Dionda* are restricted to upper waters (springs and spring-fed portions of creeks and rivers) of these drainages, generally above to the physiographic break (Balcones Escarpment) that divides the Great Plains from the Coastal Plain in the Western Gulf Slope (Conner and Suttkus, 1986) (Fig. 1A). In the Rio Grande drainage the distribution of this species complex is more complicated, including four major river systems in USA and Mexico and several small tributaries of the lower Rio Grande in both countries. South of the Rio Grande drainage, the occurrence of species in this complex is restricted to isolated populations in the upper Rio Tunal (Mezquital drainage) on the Pacific versant (Fig. 1A). All these river systems extend mainly over two physiographic provinces: the Great Plains (Pecos River and upper waters of Western Gulf drainages), and Mexican highlands (Mesa del Norte) between the Sierra Madre Oriental (Rio Salado and Rio San Juan) and the Sierra Madre Occidental (Rio Conchos and Rio Tunal).

All these species have had a confused taxonomic and systematic history (Schönhuth et al., 2008). Only *D. diaboli* has remained taxonomically stable since its original description, whereas 10 other described forms have been considered by various authors as synonyms or subspecies of *D. episcopa* (see Table 1 for summary of taxonomic background).

In the present study, we sequenced two nuclear and two mitochondrial regions from individuals sampled throughout the distribution of the genus *Dionda* (*sensu stricto*, i.e., as delimited by Schönhuth et al. (2008)) to test for monophyly, identify distinguishable and diagnosable lineages, infer phylogenetic relationships, and check for possible interspecific hybridization. We included specimens from the distribution of all six recognized and three undescribed species and followed taxonomy suggested by Mayden et al. (1992) and Schönhuth et al. (2008).

In molecular phylogenetic studies, single gene-tree analyses often show nominal species to be monophyletic for alleles at a given locus. However, at some loci, some alleles in one species may be more closely related to alleles from other species than to conspecific alleles, which can lead to erroneous evolutionary interpretations in closely related taxa (Funk and Omland, 2003). To avoid error associated with ancestral polymorphism and subsequent lineage sorting of alleles at individual loci, many authors have argued that taxonomic conclusions supported by multiple genes are preferable to conclusions derived from a single combined data matrix (e.g., Chen et al., 2003). Also, recent studies have noted the utility of comparing nuclear and mitochondrial data in identifying issues of possible hybridization, or retained ancestral polymorphisms and lineage sorting (Schönhuth and Mayden, 2010). Here, we compare separate analyses of nuclear and mitochondrial DNA sequences to delimit species of *Dionda*, and discuss relevant taxonomic and conservation implications of the results.

2. Material and methods

We analyzed sequences from 108 *Dionda* specimens collected at 69 different localities covering the distribution of all putative species of the genus, including *D. episcopa, D. diaboli, D. melanops, D. argentosa, D. serena, D. nigrotaeniata*, plus three undescribed species in Mexico (Mayden et al., 1992). These undescribed species are designated *D. sp.* [Rio Conchos], *D. sp.* [El Vergel Spring], and *D. sp.* [Ojo de Agua], and inhabit the upper Rio Conchos and two springs in the upper Rio Mezquital, in the states of Chihuahua, Zacatecas and Durango, respectively. These collections represent six independent major drainages from southwestern United States and northern Mexico: Colorado, Guadalupe/San Antonio, Nueces/Frio, Rio Grande (including Pecos, Conchos, Salado and San Juan rivers) in the Atlantic slope; a small interior basin between Durango and Zacatezas; and the Rio Tunal/Mezquital in the Pacific slope

(Fig. 1B, and Appendix A). A list of specimens examined is provided in the Appendix A. Voucher materials are deposited in ichthyological collections at Saint Louis University, St. Louis, Missouri, USA (SLU); Universidad de Nuevo León, Nuevo León, Mexico (UANL); Universidad Nacional Autónoma de Mexico, Mexico D.F. (UNAM); Museum Southwestern Biology, University of New Mexico, Alburquerque, New Mexico, USA (MSB); and University of Alabama Ichthyological Collection, Tuscaloosa, Alabama, USA (UAIC).

Four DNA regions were selected for sequencing: the complete mitochondrial cytochrome *b* gene (*cytb*, 1140 bp); the mitochondrial control region (*D-loop*, approximately 900 bp without indels); the nuclear intron S7 (*S7*, approximately 900 bp without indels); and the recombination activating gene 1 (*Rag1*, 1520 bp). DNA extraction was performed using DNeasy Tissue extraction Kit (Qiagen, Valencia, CA, USA), and ChargeSwitch gDNA Microtissue Kit (Invitrogen, Inc.). Nuclear and mitochondrial sequences were obtained from the same individuals. Amplification and primers for *cytb* are detailed in Schönhuth and Doadrio (2003); for *S7* in Chow and Hazama (1998); and for *Rag1* in López et al. (2004). For the *D-loop* region, two primers were designed for use in cyprinids (unpubl. Ph.D., Schönhuth, 2002): (DLphe) 5′-TCT TAA CAT CTT CAG TGA TAT GCT-3′ and (DLpro) 5′-CTC CCA AAG CCA GGA TTC TAA-3′. *D-loop* region PCR amplifications were carried out in a 50 μL solution containing 6 μL of DNA, 5 μL 10X PCR Ex Taq Buffer (Mg + free, 20 nM Tris–HCl, pH 8, 100 mM KCl, 0.1 mM EDTA); 0.2 μM of each primer, 0.8 mM dNTP, 2 mM of MgCl2, and 1.25 units of TaKaRa Ex Taq (TaKaRa Bio, Madison, WI, USA). After an initial denaturation step at 95 °C for 15 min, 40 cycles were performed as follows: denaturation at 94 °C (30 s), annealing at 48 °C (60 s), and extension at 72 °C (90 s), with a final extension of 5 min at 72 °C. All PCRs were carried out in a Peltier Thermal Cycler-200 (MJ Research, Waltham, MA, USA) and in GeneAmp 2700 and 9700 Thermal Cyclers (Applied Biosystems, Madrid, Spain). When more than one product resulted from PCR amplification of the *S7* region, the target product was gel-extracted and purified using a DNA Gel Extraction kit (Qiagen, Valencia, CA, USA). Primers for direct sequencing of the purified PCR were the same as those used for the PCR amplification. PCR products were sequenced at the University of Washington High-Throughput Genomics Unit (USA), and Macrogen Inc. (Korea). Sequences specifically obtained for this study have been deposited in GenBank (accession numbers **JN812338** to **JN812607**).

We used nine outgroup species (*Gila pandora, Nocomis leptocephalus, N. micropogon, N. raneyi, Campostoma pauciradii, C. ornatum, C. oligolepis, C. pullum, C. plumbeum*) in each of the independent gene analyses for D-loop, S7 and Rag1. For cytb analyses we included four additional outgroup species (*Nocomis biguttatus, N. asper, Campostoma anomalum* and *C. griseum*) to compare intrageneric divergences between currently recognized species in closely related genera.

Sequences were aligned with outgroup sequences from *G. pandora* (GP662), *C. pullum* (CP730), and *N. leptocephalus* (SN34). No ambiguous alignments or gaps were found in *cytb* or *Rag1*; therefore all codon positions were included in the analyses. Nuclear *S7* and mitochondrial *D-loop* region sequences were aligned using Clustal X ver1.85 (Thompson et al., 1997) and corrected to minimize substitutional changes. Multiple indels were detected in both regions ranging from 2 to 10 bp in *S7* and from 1 to 99 bp in the *D-loop*. For brevity observed genetic divergences mentioned herein are based on *cytb* uncorrected *p*-distances.

Phylogenetic trees were estimated separately for each data set (*cytb, D-loop, S7* and *Rag1*), as well as for the combined dataset, using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) as implemented in PAUP v4.0b10 (Swofford, 2001), RAxML (Randomized Axelerated Maximum Likelihood) v7.2.6 (Stamatakis, 2006), and Mr. Bayes v3.03
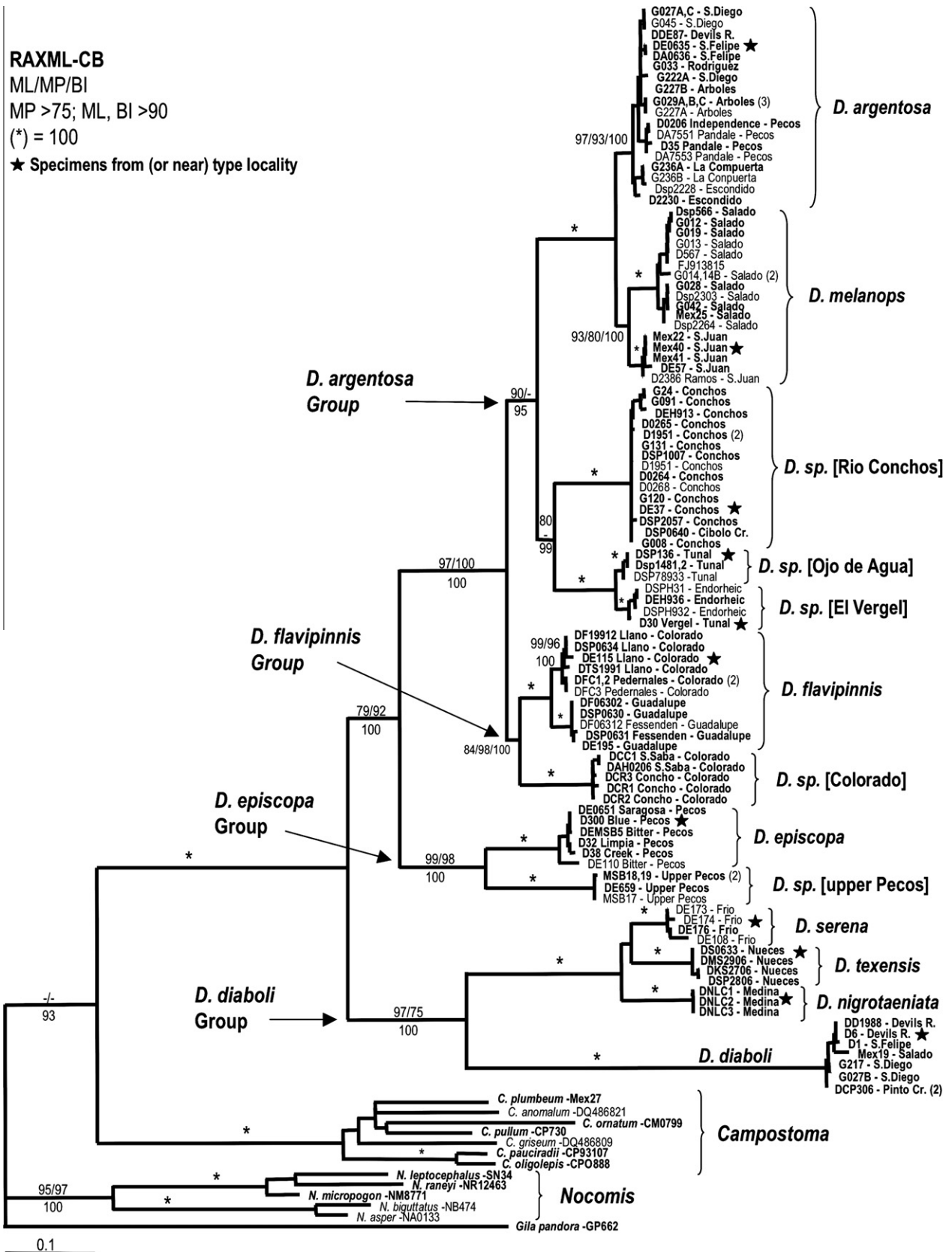
Fig. 2. Phylogenetic relationships of all *Dionda* specimens based on mitochondrial DNA regions; maximum likelihood tree (GTR + I + Γ model) for the most complete data set based on *cytb* sequences. Numbers on the branches are ML (BS > 90%) and MP (BS > 75%) bootstrap support and Bayesian posterior probabilities (PP > 90%). ∗ = all three values were 100%. Numbers in parentheses indicate identical sequences from the same locality. Stars indicate specimens from (or near) the type locality. Specimens also analyzed for the *Dloop* region are marked in bold.
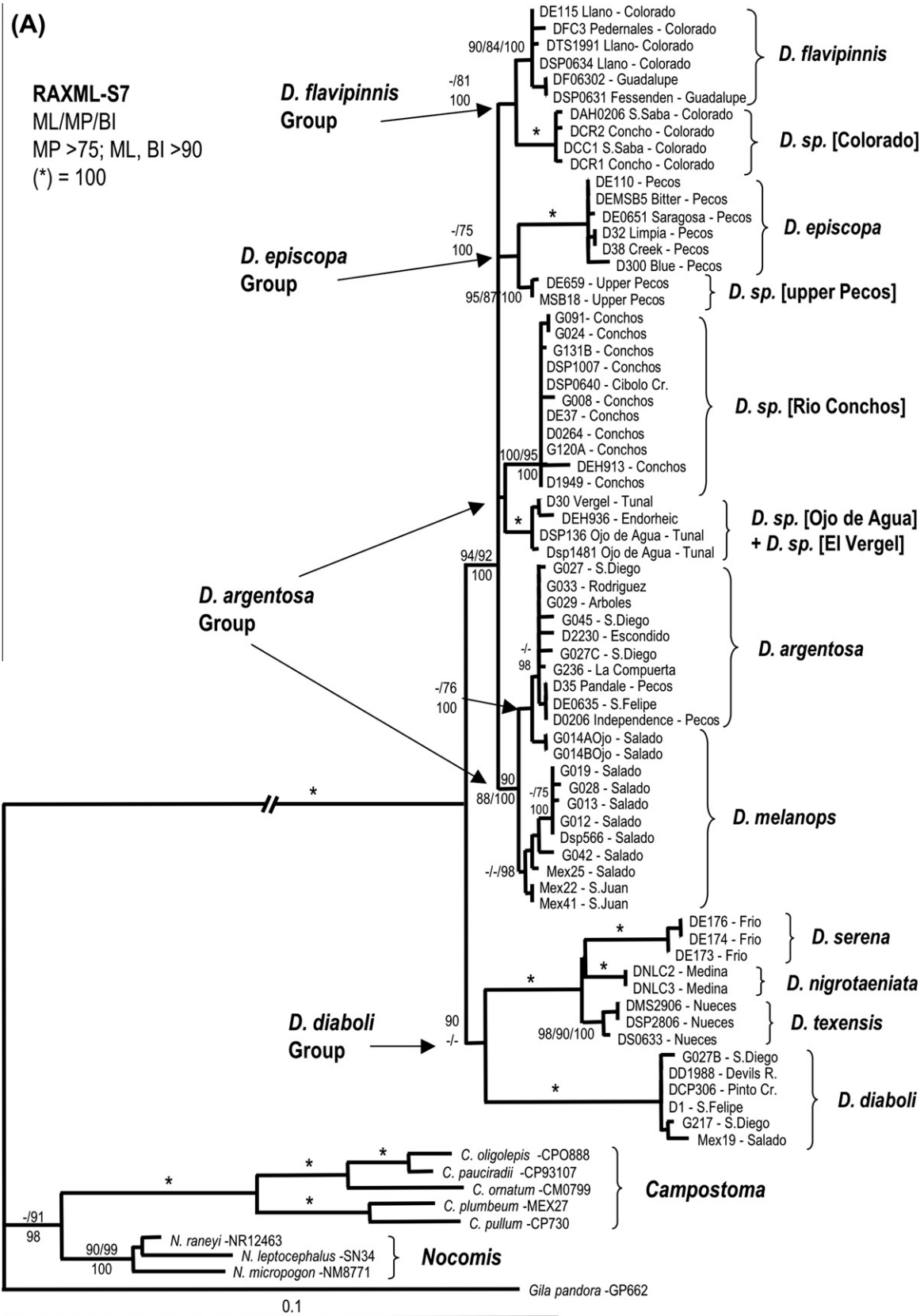
Fig. 3. Phylogenetic relationships of *Dionda* based on nuclear DNA regions. (A) Maximum likelihood tree (GTR + I + Γ model) based on sequences of intron *S7*, and (B) ML tree (GTR + I + Γ model) based on sequences of *Rag1*. Numbers on the branches are ML (BS > 90%) and MP (BS > 75%) bootstrap support and Bayesian posterior probabilities (PP > 90%). ∗ = all three values were 100%. Numbers in parentheses indicate identical sequences from the same locality.

(B)

RAXML-R1
ML/MP/BI
MP >75; ML, BI >90
(*) = 100

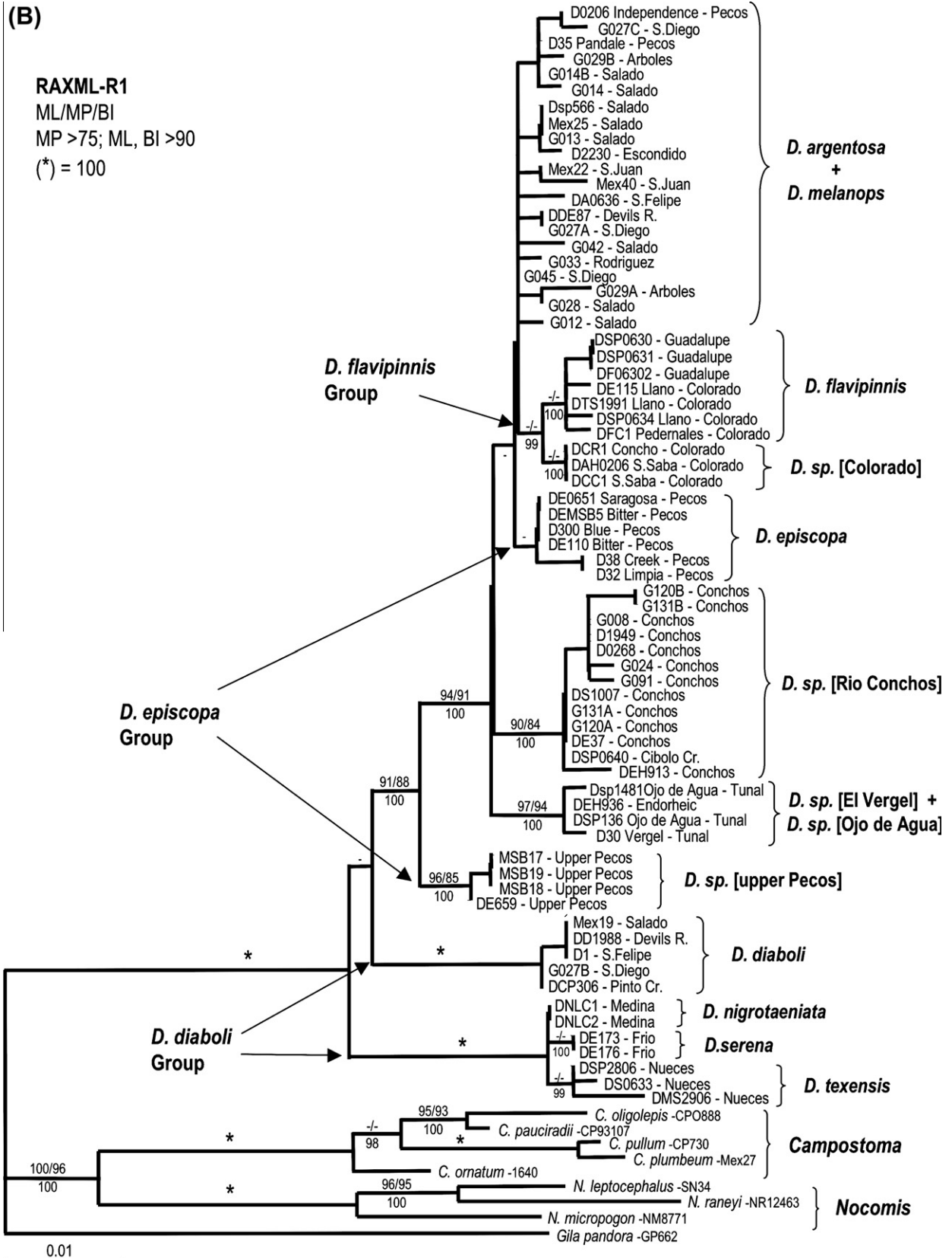Fig. 3 (*continued*)

(Huelsenbeck and Ronquist, 2001), respectively. MP analyses involved heuristic searches with ten random stepwise additions of taxa, using the MULTREE option and TBR branch swapping. The search for the optimal ML trees was conducted on a high-performance iDiscover cluster computing facility (32 nodes) located at Saint Louis University. Inferences included mixed model analyses;

partitions were assigned with respect to codon positions of the *cytb* protein-coding gene. For the ML search with the mixed model of nucleotide substitution, we used the GTR + I + G model (with four discrete rate categories). The ML tree search was conducted by performing 100 distinct runs using the default algorithm of the program for random trees (-d option) as a starting tree for each run. The final tree was determined by a comparison of likelihood scores under the GTR + I + G model among suboptimal trees obtained per run. BI analyses were conducted for each gene data set. The Akaike information criterion (AIC) implemented in MODELTEST v3.4 (Posada and Crandall, 1998) was used to choose an evolutionary model for each sequence data set. Robustness of the inferred trees was evaluated using bootstrap analysis (Felsenstein, 1985) on 1000 pseudoreplications for MP and ML. ML bootstrap results via analyses using RAxML web-servers (Stamatakis et al., 2008) were obtained from the CIPRES cluster (CIPRES portal v 2.2) at the San Diego Supercomputer Center at http://www.phylo.org/portal2/ (Miller et al., 2009). For BI, 1,000,000 generations were implemented, sampling the Markov chain at intervals of 100 generations. A total of 1000 trees (i.e., from the first 100,000 generations) were discarded as "burn-in." Support for BI tree nodes was determined based on values of Bayesian posterior probabilities obtained from a majority-rule consensus tree conducted with PAUP* (Swofford, 2001).

3. Results

We sequenced the entire cytochrome *b* gene from 108 specimens from the 69 localities sampled, whereas a representative subset of specimens from each mitochondrial clade was selected for the three other DNA regions. This resulted in a set of aligned *D-loop* sequences for 86 specimens from 63 localities (ranging from 1040 to 1144 bp), *S7* sequences from 68 specimens from 58 localities (ranging from 825 to 852 bp), and *Rag1* sequences from 73 specimens from 54 localities.

All recognized, described, or proposed new species for the genus *Dionda* (*sensu stricto*) formed a well-supported clade (Figs. 2 and 3). Almost all specimens referable to recognized or described species were recovered as monophyletic lineages. We found 12 highly supported molecular lineages including the six described and recognized species (*D. diaboli*, *D. episcopa*, *D. argentosa*, *D. melanops*, *D. serena*, and *D. nigrotaeniata*, although some of these names have been applied to the wrong species), two species that have been described but are not currently recognized (*D. flavipinnis* and

D. texensis), and four undescribed species. Each of the 12 putative species exhibited fixed diagnostic character differences (fixed diagnostic positions of nucleotides) in all or some of the four gene regions analyzed. Mitochondrial (*D-loop* and *cytb*) and nuclear (mostly *S7*) differentiation follow current taxonomic identification and were consistent with testable criteria under the phylogenetic species concept (Moritz, 1994) (Table 2).

Number of aligned sites for sequences analyzed for each DNA region, percentage of variable sites, parsimony informative sites, and range of sequence divergences (uncorrected *p*-distances) within *Dionda* for each gene are shown in Table 2. *Cytb* genetic divergences among the 12 putative species are shown in Table 3.

3.1. Mitochondrial gene analyses

We performed two separate analyses of the mtDNA sequences: one for the *cytb* gene and another for the *D-loop* region. Of the 1140 aligned sites for *cytb*, 463 sites were variable and 401 (335 without outgroups) were parsimony informative. Of the 1193 aligned sites for *D-loop*, 705 were variable and 587 (415 without outgroups) were parsimony informative. Mitochondrial *D-loop* analyses showed a higher and wider range (2.6–19.5%) of genetic divergences between lineages than did our analyses of *cytb* (1.5–16.2%). All phylogenetic analyses for both mitochondrial regions yielded concordant topologies and recovered 12 well-supported lineages with similar node support (>92% bootstrap proportions, 100% posterior probability). For brevity, we present only results recovered from the more complete data set analyses (the *cytb* gene region) (Fig. 2). Six out of these 12 lineages include six of the currently recognized species of *Dionda*, based on the inclusion of specimens from (or near) the type localities. We also identified six other well-supported and distinctive lineages of *Dionda* based on the mitochondrial analyses that are not currently recognized with species names. We consider these latter lineages as undescribed species, or propose elevation of current synonyms that are available for these species. These 12 mitochondrial lineages, consistent with species, were consistently recovered and grouped into four major well-supported species groups (see Fig. 2):

3.1.1. Dionda diaboli group

This group contains four lineages that we treat as species. One lineage includes all the populations of *D. diaboli* from several independent tributaries on both sides of the Rio Grande (Devils River, San Felipe Creek, and Pinto Creek in Texas, USA; and the upper

Table 2
Comparison of supported species of *Dionda* based on the four different DNA regions analyzed. Number of base pairs (bp) in the alignment, followed by percent variable sites, percent parsimony-informative sites, and range of divergence are indicated for each DNA region. Species supported by a given marker are indicated by " + "; species not supported are indicated by a "−".

Species	Mitochondrial DNA		Nuclear DNA	
	Cytb (1140 bp) 40.6%; 35.2%; 1.5–16.2%	**D-loop** (1193 bp) 59.1%; 49.2%; 2.3–19.5%	**S7** (952 bp) 37.5%; 20.9%; 0.3–6.5%	**Rag1** (1521 bp) 10.3%; 6.2%; 0–1.5%
D. argentosa (lower Pecos River and middle Rio Grande tributaries)	+	+	+	−
D. diaboli (middle Rio Grande tributaries)	+	+	+	+
D. episcopa (middle Pecos River)	+	+	+	−
D. flavipinnis (upper Guadalupe Dr., Llano and Pedernales rivers)	+	+	+	+
D. melanops (Rio San Juan and Rio Salado)	+	+	−	−
D. nigrotaeniata (upper Medina River)	+	+	+	−
D. serena (upper Frio River)	+	+	+	+
D. texensis (upper Nueces River)	+	+	+	+
D. sp. 1 (Rio Conchos)	+	+	+	+
D. sp. 2 (Rio Tunal)	+	+	+	+
D. sp. 3 (Northern Colorado Dr.)	+	+	+	+
D. sp. 4 (upper Pecos River)	+	+	+	+

Table 3
Summary of ranges of uncorrected p-distances for *cytb* between (below diagonal) and within (diagonal) the 12 proposed species of the genus *Dionda*. N indicates the number of specimens analyzed.

Dionda (Total N: 108)	D. argentosa	D. diaboli	D. episcopa	D. flavipinnis	D. melanops	D. nigrotaeniata	D. serena	D. texensis	D. sp. 1 [Conchos]	D. sp. 2 [Tunal]	D.sp. 3 [Colorado]	D.sp. 4 [upper Pecos]
D. argentosa (N: 21)	0–1.3											
D. diaboli (N: 8)	15.4–16.2	0–1										
D. episcopa (N: 6)	10.5–11.4	14.1–14.8	0–1.7									
D. flavipinnis (N: 12)	6.6–8.3	13.7–14.6	10.3–11.4	0–2.4								
D. melanops (N: 17)	2.4–4.2	14.2–15.5	10.5–11.4	7.2–8.1	0–2.8							
D. nigrotaeniata (N: 3)	14.9–15.1	14.5–14.9	13.1–13.5	13.4–13.9	14.5–15.4	0						
D. serena (N: 4)	13.4–14.5	13.4–14.5	12.7–13.7	12.7–14.1	13.2–14.1	4.7–5.4	0–1.4					
D. texensis (N: 4)	13.9–14.8	13.1–13.6	12.6–12.9	12.6–13.4	13.3–14.4	5.5–5.8	4.2–5.4	0–0.4				
D. sp. 1 (N: 16)	6.6–7.8	14.2–14.9	10.9–11.8	7.5–8.4	6.9–8.1	14.2–14.7	13.1–13.8	13.9–14.3	0–0.9			
D. sp. 2 (N: 8)	6.9–7.9	14.6–15	10.6–11.5	6.2–7.3	6.9–7.9	13.7–14.2	12.1–13	13.1–13.5	6.1–7	0–1.5		
D. sp. 3 (N: 5)	7.6–8.7	14.4–15	11.3–11.8	4.9–5.7	7.9–8.8	14.8–15.1	13.6–14.1	13.4–14.2	6.8–7.7	7.8–8.7	0–0.6	
D. sp. 4 (N: 4)	11.8–12.3	14.9–15.3	7.1–7.6	10.6–11	12–12.4	14.0–14.1	13.1–13.9	13.4–13.6	11.7–12.3	10.6–11.6	11.5–12	0

Rio Salado and Rio San Diego in Coahuila, MX). Three other lineages also recovered within this major clade correspond to populations from three different rivers in Texas above the Balcones Escarpment: one corresponds to populations of *Dionda* from the Frio River system (topotypic *D. serena*); the second lineage corresponds to populations in the Nueces River system (currently also referred to as *D. serena*; divergences with *D. serena* from Frio River in *cytb* ranged 4.2–5.4%); and the third lineage corresponds to *D. nigrotaeniata* in the Medina River system in Texas (see Discussion for the reasons that the name *D. nigrotaeniata* is applicable to this species). These last three closely related lineages (divergent from each other by 4.2–5.8% at *cytb*) were strongly supported as a monophyletic clade that is sister-group of *D. diaboli* (13.4–14.9% divergent at *cytb* from the other species in this group).

3.1.2. Dionda episcopa group

This group includes two highly differentiated lineages currently assigned to *D. episcopa* from the upper Pecos and middle Pecos River systems, respectively. Although these two lineages occur in the same river basin, this clade shows high interlineage genetic divergences in both mtDNA regions (*cytb*: 7.1–7.6%; *D-loop*: 8.2–8.8%). These two lineages occupy highly distinctive habitats, one in the upper Pecos River in New Mexico and the other in the isolated desert springs and spring-fed creeks of the middle Pecos River drainage in southern New Mexico and Texas. Although these two lineages have been placed by recent authors in a single species (*D. episcopa*), the strong genetic differentiation between upper and middle Pecos populations supports their recognition as two distinct species.

3.1.3. Dionda flavipinnis group

Our mitochondrial sequence analyses supported two distinct lineages from two drainages of the Western Gulf slope (Guadalupe and Colorado rivers, Texas, USA). These two sister lineages do not correspond strictly to the Guadalupe and Colorado river drainages, however. The name *D. flavipinnis* is available for populations of *Dionda* occuring throughout the Guadalupe River system as well as in the southern tributaries of the upper Colorado River system (the Llano and Pedernales drainages). Our samples of *D. flavipinnis* included specimens collected close to the type locality for this species (see Discussion for reasons that the name *D. nigrotaeniata* is not applicable to this species). The sister lineage (with divergences from *D. flavipinnis* of 4.9–5.7%) occurs in headsprings and upper reaches of the northern tributaries of the upper Colorado River (the Concho and San Saba drainages).

3.1.4. Dionda argentosa group

This group includes four lineages distributed mostly south of the Rio Grande in Mexico. The included lineages correspond to two described species (*D. argentosa* and *D. melanops*) recovered as sister species (divergences from 2.4–4.2%), and two undescribed species already identified by allozymes (Mayden et al., 1992). Ranges for these species are: (i) *D. argentosa*, including specimens from several tributaries on both sides of the Rio Grande (lower Pecos River system, Devils River, and San Felipe Creek in Texas, USA; and Rio Escondido, Rio San Diego, and Rio Rodriguez, in Coahuila, MX); (ii) *Dionda melanops*, including specimens from two large tributaries of the lower Rio Grande in Mexico, the Rio San Juan (in Coahuila) and the Rio Salado (in Nuevo León); (iii) one recognized but undescribed species, *D. sp.* [Rio Conchos], including specimens from the Rio Conchos system, in Durango and Chihuahua, MX, as well as in a small tributary of the Rio Grande (Cibolo Creek, Texas), across the Rio Grande from the mouth of the Rio Conchos; and (iv) one or two recognized but undescribed species from two isolated springs from the Rio del Tunal system, in Mexico (*D. sp.* [Ojo de Agua] and *D. sp.* [El Vergel Spring]), always recovered as
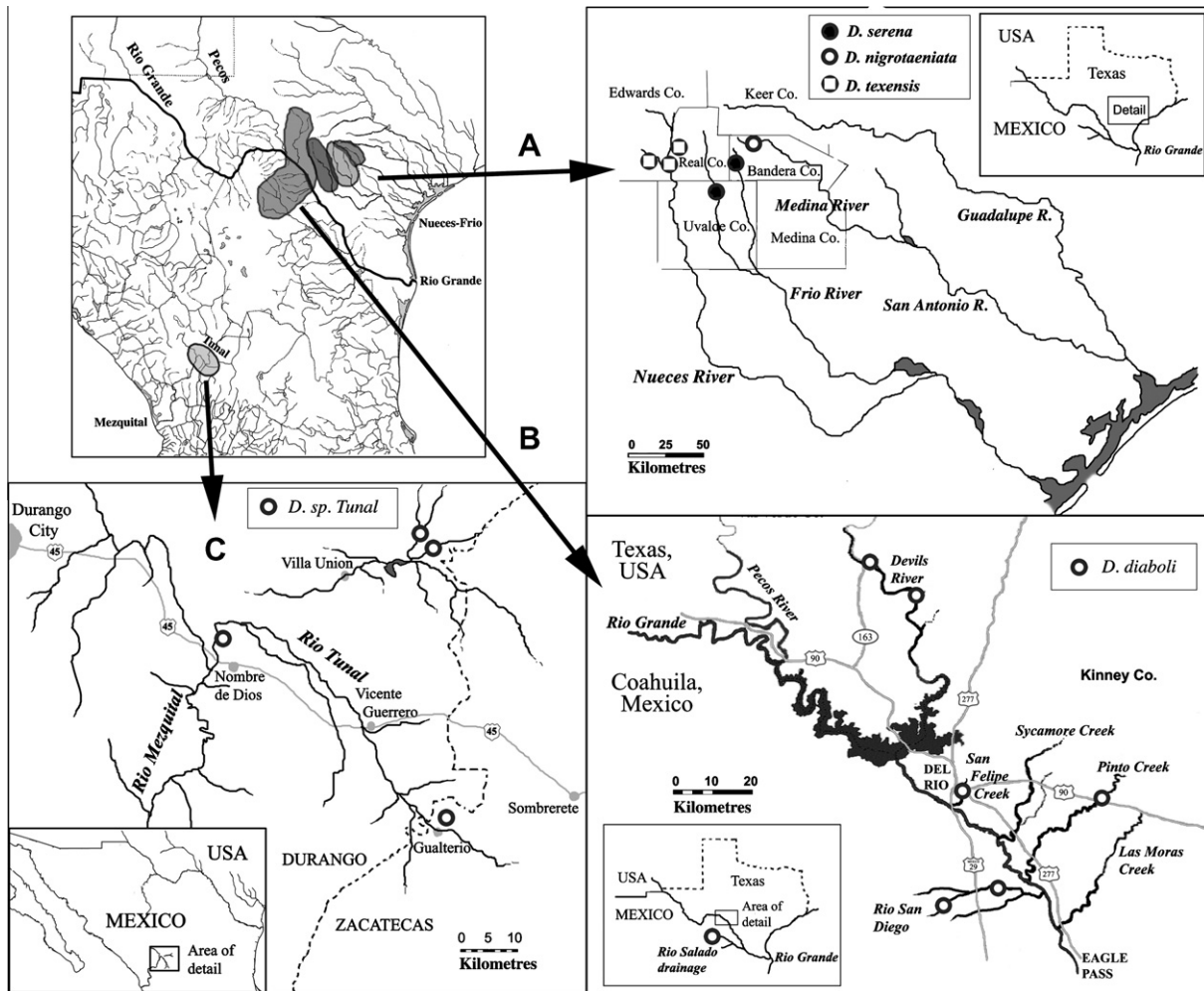
Fig. 4. Distribution of several species of *Dionda* with restricted and reduced ranges: (A) *Dionda serena, D. nigrotaeniata,* and *D. texensis;* (B) *Dionda diaboli*; and (C) *Dionda sp.* 2 [Tunal].

closely related populations in our analyses. The form that occurred in El Vergel Spring is here also reported from a small endorheic river along the Durango–Zacatecas border, MX. These two closely related lineages (*D. sp.* [Ojo de Agua] and *D. sp.* [El Vergel Spring]) exhibit the lowest levels of mitochondrial sequence divergence between any of the hypothesized species of *Dionda* (1.2–1.5%). However, they have been recognized as distinct species and reciprocally monophyletic groups on the basis of allozymes (Mayden et al., 1992). Maximum intraspecific divergences were seen among samples of *D. melanops* (2.8%). Minimum intraspecific divergences among populations were found within the undescribed species of the Rio Conchos system (0.9%), even though this species ranges widely from the upper waters of the Rio Conchos (in Durango, MX) to Cibolo Creek across the Rio Grande (in Texas, USA).

3.2. Nuclear region analyses

We performed two analyses of nuclear sequences: one for *S7* and another for *Rag1* (Figs. 3A and B). These analyses included 68–73 specimens representing all 12 mitochondrial DNA lineages found. Of the 952 aligned sites for *S7*, 357 were variable and 250 (115 without outgroups) were parsimony informative. Of the 1521 aligned sites for *Rag1*, 158 were variable and only 94 (48 without outgroups) were parsimony informative.

For the *S7* data set, all phylogenetic analyses were congruent with the mitochondrial analyses and supported most of the 12 lineages and the same interspecific relationships. However, less support for the four major clades were recovered from these nuclear sequences, with no strong support for the monophyly of the *D. argentosa* group. Also, not all specimens of *D. melanops* were supported as part of a monophyletic group in these analyses; specimens of this species from the upper Rio Salado (Ojo de Agua de Santa Maria) were more closely related to the disjunct *D. argentosa* than to the remaining conspecific populations of *D. melanops*. The remaining 11 species and major groups supported by the *S7* analyses were the same as those supported in mitochondrial sequence analyses.

Interspecific genetic divergences in the sampled nuclear regions were much smaller than those observed for the mitochondrial regions (ranging from 0.36% to 6.5% for *S7*; and from 0% to 1.54% for *Rag1*). Only 10.3% of sites vary across *Rag1* (compared to 37.5% of sites that vary across *S7*) in these comparisons. Hence, sequence divergences recovered for *Rag1* were four times lower than for *S7*. The low divergences for *Rag1* in *Dionda* logically resulted in many unresolved or poorly resolved nodes among closely related species in *Rag1* analyses. The *Rag1* analyses suggested paraphyly of the *D. diaboli* group, with *D. diaboli* more closely related to the remaining *Dionda* than to the clade composed by the three lineages above the Balcones Escarpment (Nueces, Frio and Medina rivers).
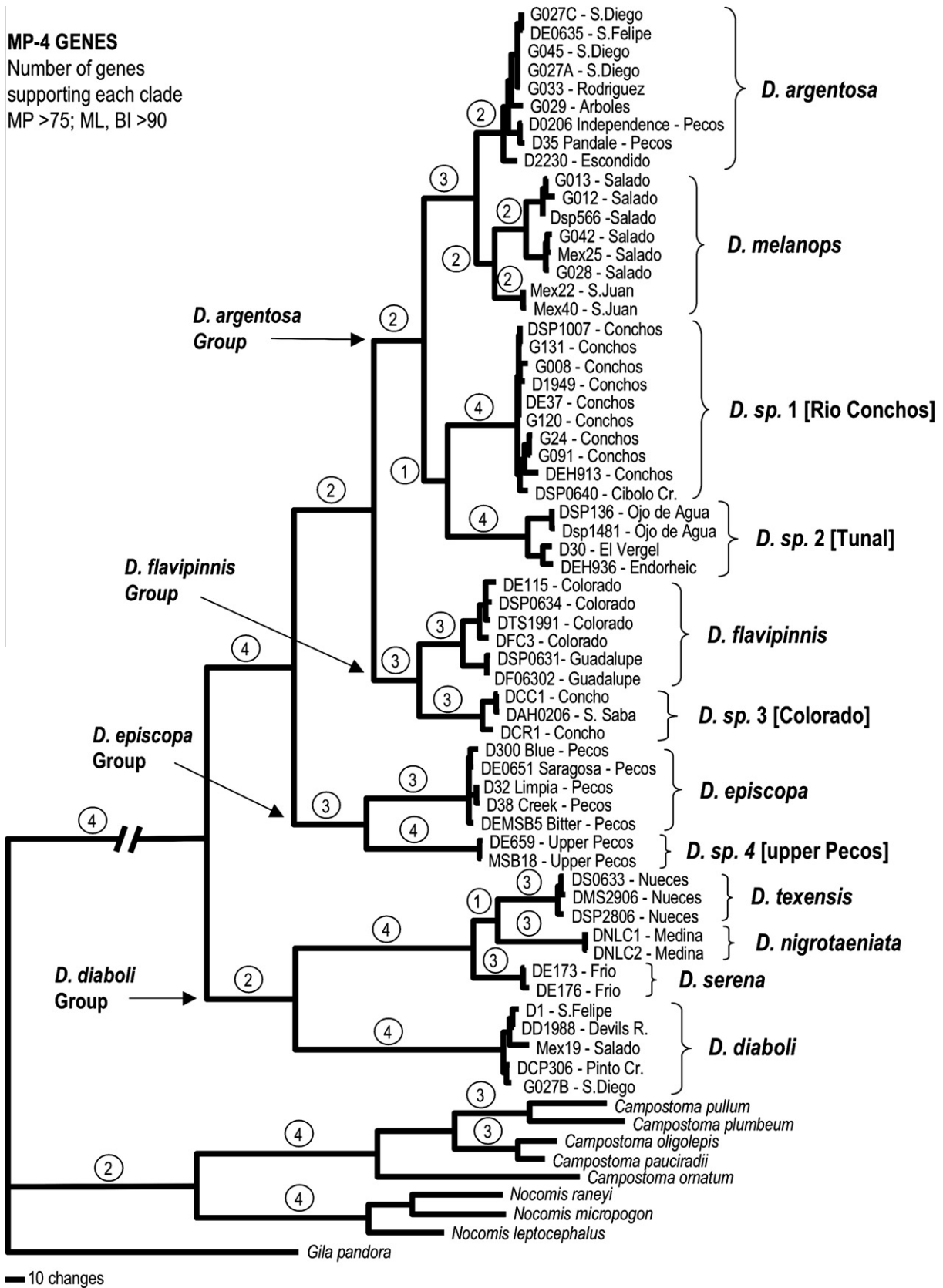
Fig. 5. Phylogenetic relationships of the 12 species of *Dionda* based on a combined MP analysis of the four DNA regions. The number of genes supporting each node are indicated within circles.

Rag1 analyses also did not resolve a sister-group relationship between populations from the upper Pecos River and the middle Pecos drainages currently assigned to *D. episcopa*. Finally, the *Rag1* analyses provided no resolution for the monoplyly of the *D. argentosa* group, nor for monophyly of *D. argentosa* and *D. melanops*.

4. Discussion

Analyses of four different DNA regions provide strong support for monophyly of the genus *Dionda* as currently recognized (Schönhuth et al., 2008). The pattern and rate of nuclear and mitochondrial sequence evolution was heterogeneous within *Dionda*, but the various DNA regions supported generally concordant lineages and relationships. Mitochondrial and nuclear data provide support for the existence of 12 distinct lineages, which we regard as species. Our results support previous suggestions based on morphology that *D. episcopa* (*sensu lato*) represents a complex of species, each of which exhibits distinctive characteristics (Hubbs and Brown, 1956). This conclusion is also consistent with allozyme analyses, which also identify a number of distinct and reciprocally monophyletic species in the *D. episcopa* complex (Mayden et al., 1992). However, our sequence analyses supported different relationships, and provided more resolution of the phylogeny of *Dionda*, compared to analyses based on allozyme variation (Mayden et al., 1992).

Both mitochondrial regions were more variable (and hence informative about phylogeny) than the two nuclear regions examined. Although *D-loop* was the most variable of the four DNA regions, and contained the most parsimony-informative characters and had the highest interspecific genetic divergences, the *cytb* gene region provided the strongest resolution of *Dionda* phylogeny. *Cytb* sequence variation provided strong support for the reciprocal monophyly of all 12 lineages proposed as species, as well as for relationships among these species. The nuclear sequences exhibited much less interspecific variation and hence provided less resolution of the relationships among these species. Despite this lower variation, *S7* (divergences 2.5 times less variable than *cytb*) provided support for the monophyly of 11 of the 12 species of *Dionda*, and *Rag1* (divergences 10.8 times less variable than *cytb*) provided support for the monophyly of 8 of the 12 species. All analyses indicated the greatest divergence between the *D. diaboli* group and the other eight species in *Dionda* (although the *Rag1* analyses suggested paraphyly of the *D. diaboli* group).

4.1. Delimiting species and taxonomic problems

The smaller effective population size of the mitochondrial genome results in fixation of haplotypes four times faster compared to nuclear loci (Moore, 1995). Previous authors have noted that this property allows resolution of isolated evolutionary lineages in many groups using mitochondrial markers that would be difficult to achieve with nuclear-based markers, including morphology (Wiens and Penkrot, 2002). However, hybridization and subsequent mitochondrial capture may produce misleading results if mitochondrial markers are used in isolation. We observed general agreement between mitochondrial and nuclear loci in this study, and also supported many of the same species that were identified in an earlier allozyme study (Mayden et al., 1992). This agreement suggests little or no hybridization among the species of *Dionda*, even in areas of current sympatry (e.g., *D. diaboli* with *D. argentosa*, or *D. diaboli* with *D. melanops*).

In this study, we considered several criteria similar to those suggested by Green (2005) to identify "genetically distinguishable species" within *Dionda*. These criteria include: (i) prior established or suggested taxonomy; (ii) inferred genetic evidence from several loci (divergences and distinctive characters between/among lineages); (iii) congruence between mitochondrial and nuclear phylogenies; and (iv) geographic congruence and range disjunction. Geographical distributions of the 12 proposed species of *Dionda* are shown in Fig. 1B, and a combined analysis of the four DNA regions is presented in Fig. 5. Here we discuss taxonomic issues involved in recognizing some of these species, and in applying available names to them (Table 1).

4.1.1. Dionda diaboli Hubbs and Brown 1956

This endangered species inhabits several independent tributaries on both sides of the lower Rio Grande in USA and Mexico. *D. diaboli* occurs in the Devils River, San Felipe Creek, and Sycamore Creek in Val Verde County, and Pinto Creek in Kinney County, in Texas, USA. Historically it was reported from Las Moras Creek, Kinney Co., but before 1980 it was eliminated from this locality (Garrett et al., 1992). There are also historical records of this species from two streams in Coahuila, MX: the Rio San Carlos and Rio Sabinas (Smith and Miller, 1986). Recently, extant populations of *D. diaboli* were confirmed in the upper Rio Sabinas (Rio Salado), MX (Schönhuth et al., 2008). In this study, we also found *D. diaboli* in the Rio San Diego in Coahuila, a small tributary of the Rio Grande across the river from Pinto Creek in Texas (Fig. 4B). This tributary is possibly the same tributary recorded as the Rio San Carlos in the literature. The specimens we analyzed from the Devils River, San Felipe Creek, Pinto Creek, Rio Salado, and Rio San Diego were always recovered in a genetically distinct and well-supported clade, with low intraspecific mitochondrial divergence (0.2–1.0%). We detected no signs of hybridization between *D. diaboli* and the sympatric species *D. argentosa* and *D. melanops*.

4.1.2. Dionda serena Girard 1856 and elevation of D. texensis Girard 1856

D. serena is currently considered to inhabit upper waters of both the Nueces and Frio rivers above the Balcones Escarpment, in Texas, USA. Specimens analyzed from these two river systems were always recovered in two reciprocally well-supported clades, but with relatively high mitochondrial divergence (4.2–5.4%) between them. *D. serena* (described from specimens from the Frio River) and *D. texensis* (described from specimens from the Nueces River) had been included in the synonymy of *D. episcopa* (Jordan and Gilbert, 1883), until Mayden et al. (1992) re-elevated and recognized *D. serena* for the combined Nueces–Frio clade. However, Mayden et al. (1992) identified two groups within *D. serena* that corresponded to the populations in the Frio and Nueces river systems, respectively. Based on mitochondrial and nuclear DNA sequence analyses, specimens from both rivers form distinct and reciprocally monophyletic clades in all the analyses. These two lineages are closely related in all the analyses, but are not always supported as sister species, as specimens from the upper Medina River (here identified as *D. nigrotaeniata*, see below) are supported in some analyses as the sister group of Frio River or Nueces River specimens. The close relationship between these three species above the Balcones Escarpment is not surprising due to close geographical proximity between upper waters of the three rivers (Medina, Nueces and Frio) (Fig. 4A). We propose *D. serena* should be restricted to populations from the upper Frio River, whereas *D. texensis* should be resurrected for populations from the upper Nueces River. The distribution of related taxa and their modes of speciation are often correlated and can be described by phylogenetic hypotheses (Wiley, 1981; Wiley and Mayden, 1985; Lynch, 1989; Funk and Brooks, 1990). *Dionda* phylogeny provides an interesting opportunity to test this expected correlation, and suggests that vicariance generated these three close related species with comparable and adjacent geographic ranges. Traditionally recognized *D. serena* (*sensu lato*: combined upper Nueces and Frio rivers populations) is listed as imperiled by Scharpf (2005) and considered to be of "special concern" in Texas waters (TWAP, 2008). However, Edwards et al. (2004) noted that *D. serena* is not federally protected and reported that it is abundant throughout its limited distribution, although its status could easily change due to reductions in water quality and/or quantity. As the range for *D. serena* is here

restricted to the upper waters of Frio River, we recommend a review of the conservation status of this species as well as for the closely related *D. texensis*.

4.1.3. Delimitation of Dionda nigrotaeniata (Cope 1880)

D. nigrotaeniata (originally named as a species of *Hybognathus*) was described from the upper waters of Wallace Creek, one of the headwaters of the Medina River, Bandera County, Texas, USA. In recent papers there is an error in the geographical location for the type locality of this species. Gilbert (1998) suggested that the Wallace Creek at which Cope (1880) collected the type series was in San Saba Co., Texas (which is in the San Saba River drainage, of the Colorado River system). However, Cope's (1880) itinerary makes it clear that he collected at the Wallace Creek in Bandera Co., Texas, a headwater creek of the Medina River system. Cope did not travel in San Saba Co. or visit the Wallace Creek there, and no *Dionda* have ever been recorded in that stream (nor have we found any specimens of *Dionda* there on repeated visits). Jordan (1885) synonymized *D. nigrotaeniata* with *D. serena*, which was later recognized as synonym of *D. episcopa*. Based on the mistaken assumptions about the type locality, Gilbert (1998) assumed that two names, *D. flavipinnis* and *D. nigrotaeniata* (both described as species of *Hybognathus* by Cope in the same paper), were available for the species of *Dionda* that occurs in the Colorado and Guadalupe River systems, as identified by Mayden et al. (1992). Using the principle of first reviser, Gilbert (1998) selected the name *D. nigrotaeniata* for this species, because the type specimens for *D. nigrotaeniata* exist but those of *D. flavipinnis* have been lost. To establish the identity of *D. nigrotaeniata*, we analyzed specimens from upper waters of the Medina River (which flows to San Antonio River), and also from upper waters of Guadalupe River and different tributaries of the Colorado Drainage. We did not locate extant populations of *Dionda* in Wallace Creek (the type locality, which has been highly impacted by development), but did locate an extant population of *Dionda* in another headwater stream of the Medina drainage (Love Creek). All analyses supported the Love Creek population of *Dionda* from the Medina drainage as closely related to *D. serena* from the Frio drainage and to *D. texensis* from the Nueces drainage, but highly divergent from *Dionda* from the Guadalupe and Colorado drainages. This result suggests that Cope (1880) was correct in recognizing *D. nigrotaeniata* (from the Medina drainage) as distinct from *D. flavipinnis* (from the Colorado drainage). Hence, *D. nigrotaeniata* appears to be the correct name for the species of *Dionda* in the headsprings of the Medina River system. Although we searched all historical localities (from literature and museum records) for *Dionda* within the Medina system, we were only able to locate a single extant population of this species (in the protected headspring of Love Creek). Most of the historical specimens of *Dionda* from the Medina drainage were collected prior to the severe drought of the 1950s, when the Medina River dried almost completely (only a few headsprings retained water). The single population of *D. nigrotaeniata* we were able to locate and analyze did not exhibit any intraspecific variation at the loci we examined. Based on the mistaken assumption that *D. nigrotaeniata* is widely distributed in the Colorado and Guadalupe drainages, the species is considered secure (Warren et al., 2000; Scharpf, 2005), and is thought to be common and abundant in good environments (Edwards et al., 2004), although the species is also considered to be of "special concern" in Texas waters (TWAP, 2008). However, with the restriction here of *D. nigrotaeniata* to the upper waters of the Medina River drainage, a reconsideration of the conservation status of this species is highly warranted.

4.1.4. Delimitation of D. episcopa Girard 1856

D. episcopa, the type species for the genus, was described from "headwaters of the Rio Pecos" and from "Camanche Spring" (a misspelling of Comanche Springs, in Fort Stockton, Pecos Co., Texas) by Girard (1856). This species is currently considered to be distributed throughout much of the Pecos River system and Rio Grande tributaries upstream of the Devils River (Mayden et al., 1992). Koster (1957) reported *D. episcopa* as one of the most abundant species in the lower portions of the Pecos River drainage in New Mexico.

Our analyses revealed considerable divergence between populations of *Dionda* in three distinct regions of the Pecos River drainage. The Pecos River originates as a clear, spring-fed river in the Sangre de Cristo Mountains of northern New Mexico (a region we refer to as the "upper Pecos"). The Pecos River then flows into a broad valley, where it becomes a low-gradient river with heavy silt loads, and gradually becomes much more saline. In the middle Pecos region in southern New Mexico and adjacent Texas, there are many springs and spring-fed creeks that originate in the Guadalupe and Davis Mountains and surrounding deserts, south and east to Comanche Springs, at Fort Stockton, Pecos Co., Texas. Although these tributaries are in the Pecos River drainage, most of these spring-fed drainages rarely reach the Pecos River on the surface, except during floods. More springs and spring-fed creeks arise in the lower Pecos region (south of Interstate Hwy. 10), beginning with Live Oak Creek, and especially Independence Creek. Independence Creek nearly matches the Pecos in flow and greatly reduces its salinity. Bonner et al. (2005) reported that "*D. episcopa*" comprised 30% of the fish assemblage in Independence Creek. Our analyses, as well as those by Carson et al. (2010), show that the *Dionda* in the lower Pecos drainage (including Independence Creek) are actually *D. argentosa*.

Given the divergence of *Dionda* populations in the upper, middle, and lower Pecos River drainage, it is important to consider the type locality of *D. episcopa*. In 1854, John Pope collected some of the type specimens used in the species description by Girard (1856) from the "headwaters of the Pecos". These specimens (USNM 00000045) are recorded as being collected in Texas. Pope collected these specimens during an expedition to survey the 32nd parallel to find a route for a railroad to the Pacific (Pope, 1854). The 32nd parallel forms the border between Texas and New Mexico. Therefore, Pope was collecting along the present Texas–New Mexico border, and the "headwaters" that Pope was referring to are the headwater tributaries of the Pecos River that flow from the Guadalupe Mountains, probably in southern New Mexico (modern Eddy Co., as suggested by Sublette et al. (1990)), or possibly in adjacent Texas. In addition, Girard (1856) lists one specific locality in the description of *D. episcopa*: specimens collected by Clark at "Camanche" [Comanche] Springs (USNM 00000046). These springs, now dry, are also in the middle Pecos drainage. Therefore, the name *D. episcopa* should be restricted to populations of the species of *Dionda* from the springs and spring-fed creeks of the middle Pecos drainage.

In the same paper in which Girard described *D. episcopa*, he also described *D. papalis* from the middle Pecos (Delaware Creek, Texas), which is just south of the Texas–New Mexico border. *Dionda papalis* was synonymized with *D. episcopa* by Jordan and Gilbert (1883). Jordan (1885) suggested that *D. papalis* was a questionable synonym of *D. serena* (which was then placed in synonymy of *D. episcopa*).

No comprehensive study has directly compared populations of *Dionda* from throughout the Pecos River drainage. Although Mayden et al. (1992) resurrected several taxa previously considered synonyms of *D. episcopa*, they did not analyze specimens from the upper Pecos River. Our data show high genetic divergence (7.2–7.5%) between the *Dionda* in the upper Pecos (at Santa Rosa, New Mexico), and the *Dionda* from springs and creeks in the middle Pecos drainage (southern New Mexico and adjacent Texas). As noted above, *D. episcopa* is the correct name for *Dionda* populations in

the creeks and springs of the middle Pecos drainage, whereas the *Dionda* in the upper Pecos (here represented by the population at Santa Rosa) are hypothesized to be an undescribed species (here referred to as *D. sp.* 4 [upper Pecos]). Intraspecific genetic divergences for *D. episcopa* (*sensu stricto*, i.e., from the middle Pecos populations) ranged from 0.1 to 1.7%. Our specimens of *D. sp.* 4 are from a single locality and we detected no intraspecific genetic variation in this population. Based on the distribution of *Dionda* shown by Sublette et al. (1990), there appears to be a wide gap between the populations in the middle Pecos in Eddy and Chaves counties, southern New Mexico, and the upper Pecos populations in De Baca and Guadalupe counties, northern New Mexico. These two areas are isolated from one another by habitat that is unsuitable for *Dionda*. It is notable that the middle Pecos streams and springs are also well isolated by the saline waters of the Pecos from the springs and spring-fed creeks of the lower Pecos, where *D. argentosa* occurs.

The traditional *D. episcopa* (*sensu lato*, i.e., including both the middle Pecos and upper Pecos forms) was at one point considered "threatened" in New Mexico (State Endangered, Group II; Cowley and Sublette, 1987), although it was removed from this list in 1983 (Sublette et al., 1990), and it is currently considered of "special concern" in Texas waters (TWAP, 2008). The conservation status of *D. sp.* 4 (upper Pecos) warrants review.

4.1.5. Resurrection of D. flavipinnis (Cope 1880)

This species was described from specimens collected in the Llano River system, which is part of the Colorado River drainage, Texas, USA. All of our analyses revealed strong support for a clade comprised of populations of *Dionda* from the Colorado and Guadalupe drainages, with two strongly supported and well-differentiated lineages within this clade. However, these two distinct lineages do not correspond strictly to populations from the two river drainages. Specimens from northern tributaries of the Colorado drainage (San Saba River and upper Concho River) formed a homogeneous and highly divergent clade, which was the sister group to a more heterogeneous clade formed by populations collected from southern tributaries of the Colorado drainage (Llano and Pedernales rivers) plus those from the Guadalupe River drainage. Many of the headwater springs of the Guadalupe River and the Llano and Pedernales rivers occur in close proximity to one another, and it is likely that headwater capture between these adjacent systems accounts for close genetic relationships of *Dionda* populations in these systems. The name *D. flavipinnis* is available for this lineage (see above for why the name *D. nigrotaeniata* is not applicable to this species). The populations from the headwaters of the northern Colorado River drainages (San Saba and Concho rivers) are highly divergent from *D. flavipinnis* (4.9–5.7% mitochondrial divergence); we consider these populations to represent a distinct, undescribed species (here referred to as *D. sp.* 3 [Colorado]). This new species is found in all our analyses to be the sister-species of *D. flavipinnnis*. The high degree of genetic divergence between *Dionda* in the southern (Llano and Pedernales) versus northern (San Saba and Concho) headwaters of the Colorado River reflects the geological and geographic isolation of these respective spring-fed systems. These two areas of headsprings (which are fed by limestone aquifers) are separated by a large area of Precambrian granite and gneiss exposures (the Llano Uplift), which contains no high-discharge springs and little potential *Dionda* habitat. The headwater habitats of *D. flavipinnis* and *D. sp.* 3 [Colorado] have been isolated since the Miocene and Pliocene erosion of limestone aquifers that once overlay the Llano Uplift (Abbott, 1975).

4.1.6. Dionda melanops Girard 1856; D. couchi Girard 1856; and D. punctifer (Garman 1881)

These three species were considered synonyms, and were all described from geographically close tributaries of the Rio San Juan,

a tributary of the lower Rio Grande drainage, in Mexico. *D. melanops* was described by Girard (1856) from Buena Vista, 8 km SE of Saltillo, in upper waters of the Rio San Juan (Coahuila, MX) (Miller, 1991). *Dionda couchi* (described in the same paper from Guajuco, Monterrey, and Caldereita, in the same river, Nuevo Leon, MX) was later synonymized with *D. melanops* (Jordan and Gilbert, 1883; Jordan, 1885; Jordan and Evermann, 1896). *Dionda punctifer* was described from specimens collected in Parras, Coahuila and a spring near Saltillo, Coahuila, MX. However, Miller (1991) showed that genus *Dionda* does not occur around Parras (Rio Nazas–Aguanaval drainage), and he suggested that all the types came from the Saltillo locality (Rio San Juan). Meek (1904) was the first to include all three species in the synonymy of *D. episcopa*. Miller (1991) examined syntypes of *D. melanops* and *D. punctifer* and found the same body shape and proportions, fin-ray counts, scale numbers, and distinctive small melanophores scattered over the body; based on these comparisons, he formally synonymized the two taxa (as *D. episcopa melanops*). Mayden et al. (1992) analyzed specimens from the Rio San Juan in Nuevo Leon and as first revisers chose and elevated *D. melanops* to species status. Miller et al. (2005) inappropriately referred to this species as *D. couchi*. *D. melanops* is currently considered to inhabit two major river systems of the lower Rio Grande (Rio San Juan and Rio Salado) in Coahuila and Nuevo Leon, Mexico (Miller et al., 2005), although no comparative analysis has been performed between populations from these two Rio Grande tributary systems. Although our sampling is inadequate for a detailed investigation of phylogeography within *D. melanops*, we detected moderate divergence of mitochondrial genomes between these two river systems (*cytb* ranging from 2.4% to 2.8%). Our analyses of nuclear loci, however, did not support the reciprocal monophyly of populations from each river drainage. Analyses of *S7* showed little divergence between populations from each river system, and specimens from an isolated spring in the Rio Salado system were supported as more closely related to *D. argentosa* than to the remaining populations of *D. melanops*. This similarity may be a result of a retained ancestral polymorphism and lineage sorting, as there appears to be no opportunity for contact between this isolated spring and populations of *D. argentosa*. *Rag1* analyses did not differentiate between *D. melanops* and *D. argentosa*. Although we provisionally consider all these populations from Rio Salado and Rio San Juan as *D. melanops*, additional analyses involving more specimens, more localities, more variable DNA regions, as well as a morphological evaluation, are warranted. Resolution of the taxonomic status of these populations is particularly important given that *D. melanops* is listed as endangered (SEDESOL, 1994).

4.1.7. Dionda argentosa Girard 1856

This species was described from specimens collected in two tributaries of the lower Rio Grande (San Felipe Creek and Devils River) in Texas, USA. *D. argentosa* was later synonymized with *D. episcopa* by Jordan and Gilbert (1883), and *D. argentosa* was regarded as a junior synonym of *D. episcopa* until Mayden et al. (1992) resurrected it. This species is thought to be restricted to rivers and creeks in Val Verde Co., Texas (Devils River, San Felipe Spring and Creek, Sycamore Creek, and in the lower Pecos River, at least as far upriver as Pandale). Here, we extend the known range for *D. argentosa* upriver in the lower Pecos to Independence Creek, Terrell Co., Texas, USA (also supported by Carson et al., 2010), and to several other small Rio Grande tributaries (Rio Escondido, Rio San Diego, Arroyo Los Arboles, and Rio Rodriguez) in Coahuila, Mexico. The range of *D. argentosa* probably includes all of the lower Pecos drainage below the spring-fed tributaries of Live Oak Creek (the surface flow of which is restricted in most years to the headsprings) and Independence Creek (which typically accounts for nearly half of the flow of the lower Pecos River below the confluence of these two systems). All these populations were

united in a homogeneous well-supported group in all but *Rag1* analyses, with small intraspecific genetic divergences (*cytb*: 0–1.3%). We found D. argentosa to be closely related to *D. melanops* in all analyses; these two species could not be clearly distinguished in the *Rag1* analyses. *D. argentosa* and *D. diaboli* are sympatric at many localities, but show no indication of hybridization. *D. argentosa* is not federally protected (Garrett et al., 1992; Cantu and Winemiller, 1997; Edwards et al., 2004). However, Edwards et al. (2004) stated that reductions in water quality could easily alter the status of this species, and it is considered a species of concern in Texas waters (TWAP, 2008).

4.1.8. Dionda sp. 1 [Rio Conchos]

Populations of *Dionda* from the Rio Conchos, in Mexico, were considered to be conspecific with *D. episcopa* until Mayden et al. (1992) recognized the Rio Conchos populations as an undescribed species. However, these authors suggested that more than one species of *Dionda* may occur in the Rio Conchos drainage. Our analyses supported populations of *Dionda* from the Rio Conchos as phylogenetically distinct from other species, but showed little genetic divergence within this species. Our samples of this undescribed species included all populations from the Rio Conchos drainage, as well as one population from a small Rio Grande tributary (Cibolo Creek) in Texas, USA, located across the Rio Grande from the mouth of the Rio Conchos (Fig. 1B). The distinctiveness of this undescribed species is supported by all the loci we examined, and it is most closely related to undescribed populations in the upper Rio Mezquital drainage (Rio Tunal), in Durango and Zacatecas, MX. This composite of undescribed species of the *D. episcopa* complex in Mexico is listed as endangered by SEDESOL (1994) and Scharpf (2005).

4.1.9. Dionda sp. 2 [Tunal]

The combined populations from the Rio Tunal drainage (an upper tributary of the Rio Mezquital system), Mexico, are supported as a distinct species in our analyses. This species has been considered to represent two undescribed forms (Mayden et al., 1992): *D. sp.* [El Vergel Spring], in El Vergel Spring near Gualterio, Zacatecas; and *D. sp.* [Ojo de Agua], from Ojo de Agua de San Juan, Durango. These two populations are not clearly divergent in our analyses, even though other data (morphology and allozymes) suggest that they be recognized as distinct species. Both of our mitochondrial analyses distinguished these two forms, but with little divergence between them; our analyses of nuclear loci did not support recognition for two different species within the Rio Tunal drainage. All our analyses placed specimens from both isolated Rio Tunal populations, as well as a newly discovered population in a small endorheic river in Durango-Zacatecas border close to Villa Union, in a well-supported and distinctive lineage (Fig. 4C). Here we consider these three isolated populations from the Rio Tunal region as one species, which we designate *D. sp.* 2 [Tunal]. This geographically isolated species occurs at the southernmost limit of the distribution of *Dionda* and it is the only species of *Dionda* that occurs on the Pacific versant. We have not located any populations of *Dionda* in the two main drainages (endorheic Rio Nazas and Rio Aguanaval river systems) between the Rio Conchos and Rio Tunal (Fig. 1B). The Rio Tunal was historically a part of the Rio Grande basin before it was captured by the Rio Mezquital system (Miller, 1991). This undescribed species probably originated with the isolation of the Rio Tunal drainage following its capture by the Rio Mezquital system, with apparent extinction of *Dionda* populations in the intervening drainages (Nazas and Aguanaval river systems). Unfortunately, we have not found this or any other native species of fishes in recent collections (February 2010) at the main bodies of El Vergel Spring or Ojo de Agua de San Juan. Additional surveys of the spring outflows and streams should be conducted to determine if remaining populations exist in the area that could be used to repopulate the main springs after exclusion of exotics (*Tilapia* and *Gambusia*). Recent collections only found *Dionda* sp. 2 [Tunal] in the small endorheic river between the states of Durango and Zacatecas. This small river is currently being used heavily for irrigation purposes, suggesting that immediate action is needed to protect this undescribed species from extinction.

Formal descriptions of the undescribed species, including comprehensive meristic and morphometrics analyses, are currently being undertaken by Mayden. Analyses of museum collections will help clarify if *D. sp.* 2 [Tunal] was originally composed of distinctive forms that have been extirpated from their original localities (El Vergel Spring and Ojo de Agua de San Juan).

5. Conclusions

We identified 12 evolutionary lineages within the genus *Dionda* (*sensu stricto*) that should be considered as basic units for taxonomy and conservation. We also (i) resurrected two species formerly considered junior synonyms (*D. flavipinnis* and *D. texensis*); (ii) circumscribed the ranges and clarified the taxonomic status of *D. episcopa*, *D. diaboli*, *D. serena* and *D. nigrotaeniata*; (iii) extended the known range of *D. argentosa* and *D. melanops*; and (iv) identified four new species that warrant formal descriptions (*D. sp.* 1 [Rio Conchos], *D. sp.* 2 [Tunal], *D. sp.* 3 [Colorado], and *D. sp.* 4 [upper Pecos]). Molecular circumscription of these four distinct undescribed lineages will facilitate their formal description. Several species of *Dionda* have been recently reduced to only one or a few extant populations, with dramatic recent loss of historical populations. A review of the conservation status of these species is needed, as several of the species appear to be critically endangered with extinction due to water loss and introduction of exotic species.

Appendix A

See Table A1.

Table A1
Sampling localities for the specimens examined in this study. Collection numbers are listed for vouchers stored at institutional collections followed by tissue numbers.

Species	Locality/drainage	Voucher (tissue)	DNA region			
			Rag1	S7	D-loop	cytb
Dionda argentosa	Rio Escondido, near Las Cuevas, Coahuila, Rio Grande Dr., MX	(D2230, D2228)	2230	2230	2230	D2230 2228
	Pecos River at Pandale, Val Verde Co., Rio Grande Dr., Texas, USA	UAIC 12755.01 (D35, DA7551, DA7553)	D35	D35	D35	D35 DA7551 DA7553
	San Felipe Spring, Moore Park, Val Verde Co., Rio Grande Dr., Texas, USA	STL 1316.03 (DA0636)	DA0636		DA0636	DA0636
	San Felipe River on Elk Lodge Property, just E of Del Rio, Val Verde Co., Rio Grande Dr., Texas, USA	STL 1315.01 (DAN0635)		DE0635	DE0635	DE0635
	Independence Creek at Hwy 349, Terrell Co., Rio Grande Dr., Texas, USA	UAIC 15362.01 (D0206)	D0206	D0206	D0206	D0206
	Devils River at Bakers Crossing, Val Verde Co., Rio Grande Dr., Texas, USA	UAIC 8354.04	DDE87	–	DDE87	DDE87
	Rio San Diego at Los Cristales, Rio San Diego, Rio Grande Dr., Coahuila, MX. 29°04′56″N 101°00′07″W	UANL 18655 (G027A, G027C) UANL19930 (G222A)	G027A G027C	G027 G027C	G027A G027C G222A	G027A G027C G222A
	Arroyo Los Arboles at Los Arboles, Rio Grande Dr., Coahuila, MX. 29°04′29″N 101°26′26″W	UANL 18621 (G029A, G029B, G029C) UANL19935 (G227A,B)	G029A G029B	G029	G029A G029B G227A,B	G029A G029B G029C G227A,B
	Spring SE of Los Alamos, Rio La Compuerta, Coahuila, MX. 28°17′18″N 101°00′11″W	UANL19548 (G236A, G236B)		G236	G236 G236B	G236 G236B
	Rio Rodriguez at El Remolino, Rio Rodriguez, Rio Grande Dr., Coahuila, MX. 28°45′40″N 101°04′28″W	UANL 18700 (G033)	G033	G033	G033	G033
	Tributary to Rio San Diego, Rio Grande Dr., Coahuila, MX. 29°07′12″N 100°57′45″W	UANL 18666 (G045)	G045	G045	G045	G045
D. diaboli	Headsprings of Pinto Creek, Kinney Co., Rio Grande Dr., Texas, USA	UAIC15358 (DCP306, DD2706)	DCP306	DC306	DC306	DCP306 DD2706
	Headsprings of San Felipe Creek, Del Rio, Val Verde Co., Rio Grande Dr., Texas, USA. 29.3656, −100.8857	STL 1096.01	D1	D1	D1	D1
	Devils River at Baker's Crossing Hwy 163, Val Verde Co., Rio Grande Dr., Texas, USA	UAIC 8354.04			D6	D6
	Rio San Juan at Las Rusias, N of Melchor Muzquiz, Rio Salado, Rio Grande Dr., Coahuila, MX	UAIC 15355.01 (Mex19)	Mex19	Mex19	Mex19	Mex19
	Devils River at Cliff Spring near Dolan Falls, Val Verde Co., Rio Grande Dr., Texas, USA	UAIC 15365 (DD1988)	DD1988	DD1988	DD1988	DD1988
	Rio San Diego at Los Cristales, Rio Grande Dr., Coahuila, MX. 29°04′56″N 101°00′07″W	UANL 18655 (G027B)	G027B	G027B	G027B	G027B
	Rio El Oregano at Rancho Los Potros, Rio Grande Dr., Coahuila, MX. 29°05′25″N 100°49′27″W	UANL19927 (G217)		G217	G217	G217
D. episcopa	Limpia Creek at Fort Davis, Jeff Davis Co., Pecos River Dr., Texas, USA	UAIC 12757.01	D32	D32	D32	D32
	Bitter Creek at Bitter Lakes, NWR 6 mi E of Roswell, Chaves Co., Pecos River Dr., New Mexico, USA	STL 110.01	DE110	DE110	–	DE110
	Sago Spring, Bitter Lake NWR, 7 mi E Roswell, Chaves Co., Pecos River Dr., New Mexico, USA	MSB 54040-05	DEMSB5	DEMSB5	DEMSB5	DEMSB5
	Creek at Highway 17, 11 km S Fort Davis, Jeff Davis Co., Pecos River Dr., Texas, USA	UAIC 12756.01 (D38)	D38	D38	D38	D38
	Blue spring, 2 mi SW of Black River Village, Eddy Co., Pecos River Dr., New Mexico, USA	STL 300.01 (DE300)	DE300	DE300	D300	DE300
	Irrigation ditch fed by Saragosa Springs, Balmorhea, Reeves Co., Pecos River Dr., Texas, USA	STL 1322.01 (DAN0651)	DE0651	DE0651	DE0651	DE0651
D. flavipinnis	Llano River at Park under Hwy 481 Loop, E of Junction, Kimble Co., Llano/Colorado River Dr., Texas, USA	STL 115.04 (DE115)	DE115	DE115	DE115	DE115
	Fessenden Spring, at Heart of the Hills Fisheries Science Center, Kerr Co., Guadalupe River Dr., Texas, USA	STL 1311.01 (DF06312)	DSP0631	DSP0631	DSP0631	DSP0631 DF06312
	Ditch and Spring at Heart of the Hills Fisheries Science Center, Kerr Co., Guadalupe River Dr., Texas, USA	(DE195)			DE195	DE195

Table A1 (*continued*)

Species	Locality/drainage	Voucher (tissue)	DNA region			
			Rag1	*S7*	*D-loop*	*cytb*
	Guadalupe River, TX Hwy 29 at Lynx Haven, Kerr Co., Guadalupe River Dr., Texas, USA	STL 1310.01 (DSP0630, DF06302)	DSP0630 DF06302	DF06302	DSP0630 DF06302	DSP0630 DF06302
	Colorado River at Municipal Park in Junction, Kimble Co., Llano/Colorado River Dr., Texas, USA	STL 1314 (DSP0634)	DSP0634	DSP0634	DSP0634	DSP0634
	Tennant Spring, 700 Spring Ranch, Kimble Co., Llano/Colorado River Dr., Texas, USA	UAIC 15366.01 (DST1991, DF19912)	DST1991	DST1991	DST1991 DF19912	DST1991 DF19912
	Flat Creek, at crossing of Hwy 290, W of Johnson City, Blanco Co., Pedernales/Colorado River Dr., Texas, USA	UAIC 15368.01 (DFC1, DFC2, DFC3)	DFC1	DFC3	DFC1 DFC2 DFC3	DFC1 DFC2 DFC3
D. melanops	Cariño de la Montaña stream, near Ejido Huizachal, Rio Salado de los Nadadores, Rio Salado, Rio Grande Dr., Coahuila, MX	(D566, D567)	566	566	566	566 567
	Rio San Juan-Sabinas near Melchor Muzquiz, Rio Salado, Rio Grande Dr., Coahuila, MX	UAIC 15355.01 (Mex25)	Mex25	Mex25	Mex25	Mex25
	Rio Sabinas, Rio Grande Dr., Nuevo León, MX	(MNCN2303)				2303
	Rio Sabinas, Rio Salado, Rio Grande Dr., Coahuila, MX	(MNCN2264)				Dsp2264
	Rio Salado de Nadadores, Rio Salado, Rio Grande Dr., Coahuila, MX. 27.034983 N–101.721443 W	UANL 17695 (G012)	G012	G012	G012	G012
	Cascada de la Madrid, Rio Salado, Rio Grande Dr., Coahuila, MX. 27°07′24″N 101°49′03″W	UANL17673 (G013)	G013	G013	G013	G013
	Ojo de Agua Santa Maria, Rio Salado, Rio Grande Dr., Coahuila, MX. 27°26′22″N 101°43′52″W	UANL 17687 (G014A, G014B)	G014 G014B	G014 G014B		G014 G014B
	Nacimiento S/N, Rio Salado, Rio Grande Dr., Coahuila, MX. 27°01′39″N 101°45′56″W	UANL 17710 (G019)		G019	G019	G019
	Rio San Juan at Puente Sabinas, Rio Salado, Rio Grande Dr., Coahuila, MX. 27°58′05″N 101°34′37″W	UANL 18299 (G028)	G028	G028	G028	G028
	Arroyo La Lajita km. 8.5 road between Musquiz-Boquillas, Rio San Juan, Rio Salado, Rio Grande Dr., Coahuila, MX. 27°57′09″N 101°34′45″W	UANL 18294 (G042)	G042	G042	G042	G042
	Rio San Juan at Castillos, Nuevo Leon, Rio Grande Dr., MX	UAIC 9158.02 (DE57)			DE57	DE57
	Rio San Juan at Allende, 74 km SE Monterrey, Rio Grande Dr., Nuevo León, MX	UAIC 15357.03 (Mex22, Mex40, Mex41)	Mex22 Mex40	Mex22 Mex41	Mex22 Mex40 Mex41	Mex22 Mex40 Mex41
	Rio Ramos, Rio San Juan, Rio Grande Dr., Nuevo León, MX	(MNCN2386)				D2386
D. nigrotaeniata	Love Creek Springs, Medina River, Bandera Co., San Antonio Dr., Texas, USA. 29.784 N–99.443 W	UAIC 15369.01 (DNLC1, DNLC2, DNLC3)	DNLC1 DNLC2	DNLC2 DNLC3	DNLC1 DNLC2 DNLC3	DNLC1 DNLC2 DNLC3
D. serena	Can Creek, Lost Maples State Park, Bandera Co., Sabinal River, Frio River Dr., Texas, USA	UAIC 8348.02 (DE173-5)	DE173 DE176	DE173 DE174 DE176	DE175 DE176	DE173 DE174 DE176
	Frio River at Hwy 127, Uvalde Co., Frio River Dr., Texas, USA	(DE108)				DE108
D. texensis	Paisano Spring, Edwards Co., Nueces River Dr., Texas, USA	UAIC 15359.01 (DSP2806)	DSP2806	DSP2806	DSP2806	DSP2806
	Kickapoo Spring, Edwards Co., Nueces River Dr., Texas, USA	UAIC 15361.01 (DK2706)			DK2706	DK2706
	Morris Spring, Real Co., Nueces River Dr., Texas, USA	UAIC 15360.01 (DMS2906)	DMS2906	DMS2906	DMS2906	DMS2906
	Nueces River at Texas Hwy. 55 in Barksdale, Real/Edwards Co., Nueces River Dr., Texas, USA	STL 1313 (DS0633)	DS0633	DS0633	DS0633	DS0633
D. sp. 1 (Rio Conchos, Durango and Chihuahua, MX)	Rio San Juan, 26 km S Canutillo on Hwy 45, Rio Florido, Rio Conchos Dr., Durango, MX	UAIC 7904.01 (D23)	DE37	D23	D23	DE37, D23
	Rio Conchos Dr., Durango, MX	(D1949-51)	1949	1949	1949-50	1949-51
	Isolated pool at Arroyo de los Alcores, Rio Conchos Dr., Chihuahua, MX	(D0264)		D0264	D0264	D0264
	Rio Nonoava at Nonoava, Rio Conchos Dr., Chihuahua, MX	(D0265)			D0265	D0265

Table A1 (*continued*)

Species	Locality/drainage	Voucher (tissue)	DNA region			
			Rag1	S7	D-loop	cytb
	Rio Conchos, Rio Conchos Dr., Chihuahua, MX	UAIC 14967.03 (D0268)	D0268			D0268
	Cibolo Creek at Shafter, Presidio Co., Rio Grande Dr., Texas, USA	STL1320.01 (DSP0640)	DSP0640	DSP0640	DSP0640	DSP0640
	Rio Florido at Villa Coronado, Rio Conchos Dr., Chihuahua, MX 26°44′06″N 105°03′55″W	UANL 16116 (G008) UANL 19035 (G131A, B)	G008 G131A,B	G008 G131B	G008 G131A	G008 G131A
	La Boquilla at Tres Ojitos, Arroyo Carretas, Rio San Pedro, Rio Conchos Dr., Chihuahua, MX. 28°18′37″N 106°41′09″W	UANL 18516 (G024)	G024	G024	G024	G024
	Rio San Pedro at Satevo, Rio Conchos Dr., Chihuahua, MX 29°20′03″N 104°55′15″W	UANL 19063 (G091)	G091	G091	G091	G091
	Rio Santa Isabel, road from Cuauhtemoc to Chihuahua at km 46, Rio Conchos Dr. Chihuahua, MX. 28 20′13.3″N 106 21′18.8″W	IBUNAM-P15689 (DEH913)	DEH913	DEH913	DEH913	DEH913
	Rio Conchos at Hidalgo del Parral, Rio Conchos Dr., Chihuahua, MX 26°54′48″N 105°45′46″W	UANL 19010 (G120A)	G120A G120B	G120A	G120	G120A
	Upper Rio Florido, Rio Conchos Dr., Chihuahua, MX	STL 15376.05 (DSP1007, DSP2057)	DSP1007	DSP1007	DSP1007 DSP2057	DSP1007 DSP2057
D. sp. 2 (Rio Tunal Dr., Durango, and Zacatecas, MX)	Ojo de Agua de San Juan, 1 km N of Los Berros, Nombre de Dios, Rio Tunal, Rio Mezquital Dr., Durango, MX	STL 255.01 UAIC7893.01 (D1481-1482)	D136 1481	D136 1481	D136 1481	D136 DSP78933 14,811,482
	El Vergel Spring, near Gualterio, Rio del Tunal, Rio Mezquital Dr., Zacatecas, MX	UAIC 7894.01	D30	D30	D30	D30
	Rio Caliente, upstream from Presa Francisco Villa, Endorheic Dr., Durango, MX. 24 01′16.4″N 103 54′37.6″W; alt. 2038 m	IBUNAM-P15770 (DSPH932, DSPH31)				DSPH932 DSPH31
	Rio La Saucera, upstream from Presa Francisco Villa, Endorheic Dr., Durango, MX. 24 00′41.0″N 103 53′98.5″W; alt. 2004 m	(DEH936)	DEH936	DEH936	DEH936	DEH936
D. sp. 3 (North Colorado Dr., Texas, USA)	Headsprings of Clear Creek, Menard Co., San Saba River, Colorado River Dr., Texas, USA	UAIC 15363.01 (DCC1)	DCC1	DCC1	DCC1	DCC1
	Clear Creek Spring, Menard Co., San Saba River, Colorado River Dr., Texas, USA	UAIC 15364.01 (DAH0206)	DAH0206	DAH0206	DAH0206	DAH0206
	Upper Concho River at Cristobal, Tom Green Co., Colorado River Dr., Texas, USA	UAIC 15367.01 (DCR1, DCR2, DCR3)	DCR1	DCR1 DCR2	DCR1 DCR2 DCR3	DCR1 DCR2 DCR3
D. sp. 4 (upper Pecos River, New Mexico, USA)	El Rito Creek, 1 mi S of Santa Rosa, Guadalupe Co., Pecos River Dr., New Mexico, USA	STL 659.02 MSB 54054-18 MSB 54054-17 MSB 54054-19	DE659 MSB18 MSB17 MSB19	DE659 MSB18	DE659 MSB18 MSB19	DE659 MSB18 MSB17 MSB19

Outgroups

Species	Locality/drainage	Voucher (tissue)	Rag1	S7	D-loop	cytb
Gila pandora	Rio Chama at US Hwy 84 near Arlequín, Rio Arriba Co.,New Mexico, USA	STL662.01	GP662	GP662	GP662	GP662
Nocomis leptocephalus	Buffalo River, Wilkinson Co., Mississippi, USA	UAIC 11555.01	SN34	SN34	SN34	SN34
Nocomis raneyi	Dan River at public access off of NC Hwy 8/89, 2.3 mi N of Hanging Rock, Strokes Co., North Carolina, USA	UAIC 12463.02 (NR12463)	NR12463	NR12463	NR12463	NR12463
Nocomis micropogon	Red Bird River, K4 MI 1-6, Kentucky River drainage, Clay Co., Kentucky, USA	UAIC 7972.05 (NM8771)	NM8771	NM8771	NM8771	NM8771
Nocomis biguttatus	Little Saline Creek at MO Hwy N just S of Ozora, St., Genevieve, Missouri, USA	STL 1344.02 (NB474)				NB474
Nocomis asper	Elk River at AR Hwy 59 in Sulphur Springs, Bemton Co., Arkansas, USA	UAIC 12549.01 (NA0133)				NA0133
Campostoma pullum	Meramec River at MDC access at MO Hwy 8, Cranford Co., Missouri, USA	STL 730.02	CP730	CP730	CP730	CP730
Campostoma	Emory River at Deermont Rd. in Camp Austin, Morgan	STL 888.02	CPO888	CPO888	CPO888	CPO888

Table A1 (*continued*)

Species	Locality/drainage	Voucher (tissue)	DNA region			
			Rag1	S7	D-loop	cytb
oligolepis	Co., Mississippi Dr., Tennessee, USA					
Campostoma ornatum	Rio Yepachic at Rancho Santiago just N of Yepachic, Rio Mulatos, Papigochic, Yaqui Dr., Chihuahua, MX	UAIC 14280. 01 (CM0799)	1640	CM0799	CM0799	CM0799
Campostoma pauciradii	Snake Creek, 12.2 miles N County line, Russell Co., Alabama, USA	UAIC 10858.01 (CPO93107)	CPO93107	CPO93107	CPO93107	CPO93107
Campostoma plumbeum	Rio San Juan, Allende, 74 km SE Monterrey, Rio Grande Dr., Nuevo Leon, MX	UAIC 15406.01 (Mex27)	Mex27	Mex27	Mex27	Mex27
Campostoma anomalum	GenBank Sequence KY, Nelson Co., Rolling Fork River, USA	UAIC10147.02				DQ486821
Campostoma griseum	GenBank Sequence AR, Montgomery Co., Caddo River, USA	SLUM 423.02				DQ486809

Dr.: Drainage; USA: United States of America; MX: Mexico; STL: Saint Louis University, St. Louis, Missouri, USA; UAIC: University of Alabama Ichthyological Collection, Tuscaloosa, Alabama, USA; UANL Universidad Autónoma de Nuevo León, Nuevo León, MX; IBUNAM: Universidad Nacional Autónoma de México, México DF, MX; MSB: Museum Southwestern Biology, Alburquerque, New Mexico, USA.

References

Abbott, P.L., 1975. On the hydrology of the Edwards Limestone, south-central Texas. J. Hydrol. 24, 251–269.

Agapow, P.-M., Bininda-Emonds, O.R.P., Crandall, K.A., Gittleman, J.L., Mace, G.M., Marshall, J.C., Purvis, A., 2004. The impact of species concept on biodiversity studies. Q. Rev. Biol. 79, 161–179.

Bonner, T.H., Thomas, C., Williams, C.S., Karges, J.P., 2005. Temporal assessment of a west Texas stream fish assemblage. Southwest. Nat. 50, 74–106.

Cantu, N.E.V., Winemiller, K.O., 1997. Structure and habitat associations of Devils River fish assemblages. Southwest. Nat. 42, 265–278.

Carson, E.W., Hanna, A.H., Garrett, G.P., Gibson, J.R., Gold, J.R., 2010. Conservation genetics of cyprinid fishes (genus *Dionda*) in southwestern North America. II. Expansion of the known range of the Manantial Roundnose minnow, *Dionda argentosa*. Southwest. Nat. 55, 576–581.

Chen, W.-J., Bonillo, C., Lecointre, G., 2003. Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. Mol. Phylogenet. Evol. 26, 262–288.

Chow, S., Hazama, K., 1998. Universal PCR primers for S7 ribosomal protein gene introns in fish. Mol. Ecol. 7, 1255–1256.

Conner, J.V., Suttkus, R.D., 1986. Zoogeography of freshwater fishes of the western Gulf Slope of North America. In: Hocutt, C.H., Wiley, E.O. (Eds.), The Zoogeography of North American Freshwater Fishes. Wiley, New York, pp. 413–456.

Cope, E.D., 1880. On the zoological position of Texas. Bull. U.S. Natl. Mus. 17, 1–51.

Cowley, D.E., Sublette, J.E., 1987. Distribution of fishes in the Black River drainage, Eddy County, New Mexico. Southwest. Nat. 32, 213–221.

Edwards, R.J., Garrett, G.P., Allan, N.L., 2004. Aquifer-dependent fishes of the Edwards Plateau region. In: Mace, R.E., Angle, E.S., Mullican, W.F. (Eds.), Aquifers of the Edwards Plateau. Texas Water Development Board, pp. 253–268 (Chapter 13).

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Funk, V.A., Brooks, D.R., 1990. Phylogenetic Systematics as the Basis of Comparative Biology. Smithsonian Inst. Press, Washington, DC, 45 pp.

Funk, D.J., Omland, F.E., 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34, 397–423.

Garrett, G.P., Edwards, R.J., Price, A.H., 1992. Distribution and status of the Devils River minnow, *Dionda diaboli*. Southwest. Nat. 37, 259–267.

George, A.L., Mayden, R.L., 2005. Species concepts and the Endangered Species Act: how a valid biological definition of species enhances the legal protection of biodiversity. Nat. Resour. J. 45, 369–407.

Gilbert, C.R., 1998. Type catalog of recent and fossil North American freshwater fishes: families Cyprinidae, Catostomidae, Ictaluridae, Centrarchidae, and Elassomatidae. Fla. Mus. Nat. Hist. Spec. Publ. 1, 1–284.

Girard, C., 1856. Researches upon the cyprinid fishes inhabiting the fresh waters of the United States of America west of the Mississippi Valley, from specimens in the museum of the Smithsonian Institution. Proc. Acad. Nat. Sci. Phil. 8, 165–213.

Green, D.M., 2005. Designatable units for status assessment of endangered species. Conserv. Biol. 19, 1813–1820.

Hubbs, C., Brown, W.H., 1956. *Dionda diaboli* (Cyprinidae), a new minnow from Texas. Southwest. Nat. 1, 69–77.

Huelsenbeck, J.P., Ronquist, F.R., 2001. Mr. Bayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Jordan, D.S., Evermann, B.W., 1896. The fishes of North and Middle America: a descriptive catalogue of the species of fish-like vertebrates found in the waters of North America, north of the Isthmus of Panama. Bull. U.S. Natl. Mus. 47, 1–1240.

Jordan, D.S., Gilbert, H.C., 1883. Synopsis of the fishes of North America. Bull. U.S. Natl. Mus. 16, 1–1018.

Jordan, D.S., 1885. Identification of the fishes of Cyprinidae and Catostomidae, described by Dr. Charles Girard, in the Proceedings of the Academy of Natural Sciences of Philadelphia for 1856. U.S. Natl. Mus. 8, 118–127.

Koster, W.J., 1957. Guide to the fishes of New Mexico. Univ. New Mexico Press, Albuquerque, 116pp.

López, J.A., Chen, W.-J., Ortí, G., 2004. Esociform phylogeny. Copeia 2004 (3), 449–464.

Lynch, J.D., 1989. The gauge of speciation: on the frequency of modes of speciation. In: Otte, D.H., Endler, J.A. (Eds.), Speciation and Its Consequences. Sinnauer, Sunderland, MA, pp. 527–553.

Mayden, R.L., Matson, R.H., Hillis, D.M., 1992. Speciation in the North American Genus *Dionda* (Teleostei: Cypriniformes). In: Mayden, R.L. (Ed.), Systematics, Historical Ecology and North American Freshwater Fishes. Standford Univ. Press, California, pp. 710–746.

Meek, S.E., 1904. The fresh-water fishes of Mexico north of the Isthmus of Tehuantepec. Field Columbian Mus. Zool. Ser. 5, 1–252.

Miller, R.R., 1991. Taxonomic status of two nominal species of Mexican freshwater fishes (Cyprinidae). Copeia 1991 (1), 225–227.

Miller, R.R., Minkley, W.L., Norris, S.M., 2005. Freshwater Fishes of Mexico, first ed. University of Chicago Press, Chicago, 490pp.

Miller, M.A., Holder, M.T., Vos, R., Midford, P.E., Liebowitz, T., Chan, L., Hoover, P., Warnow, T., 2009. The CIPRES Portals. CIPRES. Archived by WebCite(r). <http://www.webcitation.org/5imQlJeQa>. <http://www.phylo.org/sub_sections/portal> (accessed 04.08.09).

Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49, 718–726.

Moritz, C., 1994. Applications of mitochondrial DNA in conservation: a critical review. Mol. Ecol. 3, 403–413.

Pope, J., 1854. Reports of Explorations and Surveys to Ascertain the Practicable and Economical Route for A Railroad from the Mississippi River to the Pacific Ocean Made Under the Direction of the Secretary of War, in 1854–1855, According to Acts of Congress of March 3, 1853 May 31, 1854, and August 5. 1854, vol. 6, 33rd Congress, 2nd Session, Senate Exe. Doc. No. 78, Washington, DC.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the models of DNA substitution. Bioinformatics 14, 817–818.

Rojas, M., 1992. The species problem and conservation: what are we protecting? Conserv. Biol. 6, 170–178.

Scharpf, C., 2005. Annotated checklist of North American freshwater fishes including subspecies and undescribed forms, Part 1: Petromyzontidae through Cyprinidae. Am. Curr. Spec. Publ. 31, 1–44.

Schönhuth, S., 2002. Sistemática, filogenia e implicaciones biogeográficas del género *Notropis* Rafinesque, 1817 (Actinopterygii: Cyprinidae) en el centro y sur de México. Ph. Diss., Universidad Complutense de Madrid, Spain.

Schönhuth, S., Doadrio, I., 2003. Phylogenetic relationships of Mexican minnows of the genus *Notropis* (Actinopterygii: Cyprinidae). Biol. J. Linn. Soc. 80, 323–337.

Schönhuth, S., Mayden, R.L., 2010. Phylogenetic relationships in the genus *Cyprinella* (Actinopterygii: Cyprinidae) based on mitochondrial and nuclear gene sequences. Mol. Phylolgenet. Evol. 55, 77–98.

Schönhuth, S., Doadrio, I., Mayden, R.L., 2006. A biogeographic perspective on the phylogeny of Mexican cyprinids (Actinopterygii, Cyprinidae). In: Lozano-Vilano, M.L., Contreras-Balderas, A.J. (Eds.), Studies of North American Desert Fishes in Honor of E.P. (Phil) Pister, Conservationist. Universidad Autónoma de Nuevo León, México, pp. 102–124.

Schönhuth, S., Doadrio, I., Dominguez, O., Hillis, D.M., Mayden, R.L., 2008. Molecular evolution of Southern North American Cyprinidae (Actinopterygii), with the

description of the new genus *Tampichthys* from central Mexico. Mol. Phylogenet. Evol. 47, 729–756.

SEDESOL, 1994. México: Informe de la situación general en material de equilibrio ecológico y protección al ambiente 1993–1994. SEDESOL, México D.F., 376pp.

Smith, M.L., Miller, R.R., 1986. The evolution of the Rio Grande Basin as inferred from its fish fauna. In: Hocutt, C.H., Wiley, E.O. (Eds.), The Zoogeography of North American Freshwater Fishes. Wiley-Interscience Publications, New York, pp. 457–485.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A fast bootstrapping algorithm for the RAxML web-servers. Syst. Biol. 57, 758–771.

Sublette, J.E., Hatch, M.D., Sublette, M., 1990. The Fishes of New Mexico. University of New Mexico Press, Albuquerque, 393pp.

Swofford, D.L., 2001. PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods) Version 4.0b10. Sinauer, Sunderland, MA.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface. Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 634–640.

TWAP, 2008. Texas Wildlife Action Plan. <http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_pl_w7000_1187a/>.

Waples, R.S., 1998. Evolutionarily significant units, distinct populations segments, and the endangered species act: reply to Pennock and Dimmick. Conserv. Biol. 12, 718–721.

Waples, R.S., Adams, P.B., Bohnsack, J., Taylor, B.L., 2007. A biological framework for evaluating whether a species is threatened or endangered in a significant portion of its range. Conserv. Biol. 21, 964–974.

Warren Jr., M.L., Burr, B.M., Walsh, S.J., Bart Jr., H.L., Cashner, R.C., Etnier, D.A., Freeman, B.J., Kuhajda, B.R., Mayden, R.L., Robison, H.W., Ross, S.T., Starnes, W.C., 2000. Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. Fisheries, Conservation 25, 7–29.

Wiens, J.J., Penkrot, T., 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). Syst. Biol. 51, 69–91.

Wiley, E.O., 1981. Phylogenetics. The Theory and Practice of Phylogenetic Systematics. J. Wiley and Sons, New York, 439pp.

Wiley, E.O., Mayden, R.L., 1985. Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. Ann. Missouri Bot. Gard. 72, 596–635.