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Molecular Phylogenetics and Evolution 47 (2008) 729–756
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Molecular evolution of southern North American Cyprinidae (Actinopterygii), with the description of the new genus *Tampichthys* from central Mexico

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Received 16 August 2007; accepted 26 November 2007
Available online 1 February 2008

Abstract

Most of the recognized species of the genus *Dionda* inhabit drainages of the Gulf of Mexico from central Mexico to central Texas, USA, and have been considered a monophyletic group based on morphological, osteological, and allozyme investigations. Phylogenetic relationships of 15 species of *Dionda* and 34 species from closely related genera were inferred from one mitochondrial (cyt*b*) and three nuclear gene sequences (S7, Rhodopsin, Rag1) totaling 4487 nucleotides. Separate analyses of all four genes yield congruent phylogenies; however the 15 putative species of *Dionda* evaluated were never recovered as a monophyletic group when species from nine related genera were included in the analyses. Among the ingroup taxa, one well-supported and highly divergent clade is consistently recognized and consists of six recognized and three undescribed northern species currently recognized in the genus *Dionda*. These nine species inhabit present or past tributaries of the Rio Grande basin of northern Mexico and southern USA, and were recovered as a basal clade in all analyses. Another large, also strongly supported clade, consisting of seven genera, include five southern recognized species currently in the genus *Dionda*, forming the sister group to the *Codoma* clade. These five species comprise the "Southern *Dionda* clade" and inhabit headwaters of the Pánuco–Tamesí drainage and some adjacent coastal rivers in the Tampico Embayment. The consistent and repeated identification of eight different clades recovered in most of the separate gene analyses strongly supports a division of the non-natural genus *Dionda*. A new genus, *Tampichthys*, is proposed for the clade of species endemic to east-central Mexico and formerly in *Dionda*. *Tampichthys* and the putative monotypic genus *Codoma* are more related to Mexican species of the genera *Cyprinella* and *Notropis* than to other species referred to *Dionda* sensu stricto.

Keywords: Systematics; Cyprinidae; *Dionda*; Phylogeny; Nuclear DNA; Mitochondrial DNA; Mexico; Speciation

1. Introduction

The neotropical cyprinid genus *Dionda*, originally described by Girard (1856), currently includes 12 described species (*D. episcopa*, *D. argentosa*, *D. serena*, *D. diaboli*, *D. melanops*, *D. ipni*, *D. erimyzonops*, *D. mandibularis*, *D. dichroma*, *D. catostomops*, *D. nigrotaeniata* and *D. rasconis*), with at least four more undescribed species supported by both morphological (Miller and Mayden, unpub.) and allozyme analyses (two from Río Tunal, upper Mezquital drainage; one from Río Conchos, Rio Grande Drainage; and one from Río Axtla, Pánuco drainage) (Mayden et al., 1992). Most of the species included in the present genus *Dionda*

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doi:10.1016/j.ympev.2007.11.036

occur in drainages of the Gulf of Mexico from the Colorado River in the Western Gulf Slope, Texas, USA, to the Río Misantla in Veracruz, Mexico. From east to west and south, drainages currently known to contain species of *Dionda* include the Colorado, Guadalupe/San Antonio, Nueces/Frio, Rio Grande, Mezquital, Tamesí, Pánuco and some coastal rivers south of the Río Pánuco drainage (Fig. 1). Species of *Dionda* are restricted to headwater habitats (springs, streams and Creeks) of these drainages, in clear, cool waters over a gravel or sand/gravel substratum. Only *D. ipni* also is known to occur also in coastal rivers of the Gulf of Mexico, south of the Río Pánuco drainage.

In general, the standard size, external appearance and internal morphology of this group of fishes are similar and all these species share a series of common characteristics. However, species within this genus also present unique characters, have been known to span a broad range of habitats, and in most of these species there is a high degree of variation in the development of barbels and the intestinal coiling, as occurs with genus *Algansea* and central and southern Mexican *Notropis* (Hubbs and Miller, 1977). These last two characteristics have been argued to be putatively diagnostic generic criteria for some North American genera that have been critically examined using phylogenetic methods (Mayden, 1989). Interestingly, these two traits break down in Mexican cyprinids (Hubbs and Miller, 1977), yet the monophyly of this genus has not been debated or in question, probably because the species are similar in external morphology and size.

1.1. Phylogenetic relationships overview

This genus comprises a group of species with a controversial taxonomic history. For a long time, species now placed in *Dionda* were considered as part of genus *Hybognathus* Agassiz. Jordan (1924) stated that "*Dionda* differ from *these silvery forms* that have longer and less hooked teeth". Later, in the description of two new species (*D. catostomops*, *D. dichroma*) Hubbs and Miller (1977) differentiated six southern species in east-central Mexico from

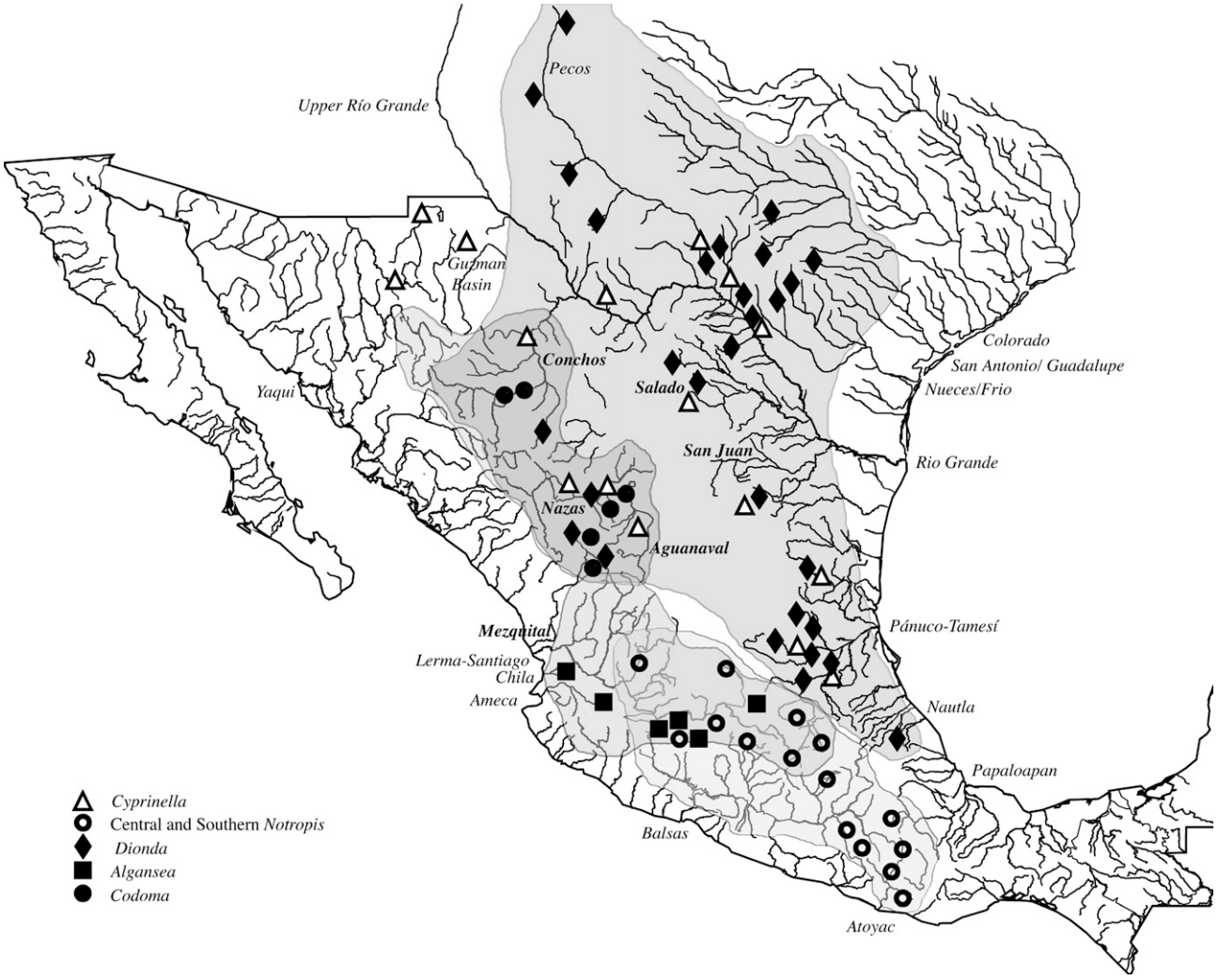

Fig. 1. Localities from which specimens of different genera and species were sampled (see Appendix A). Distribution of each genus is represented in different grey patterns. Main drainages sampled are also indicated.

Hybognathus. However, Hubbs and Miller (1977) discussed the problem of recognizing and delimiting this genus. Upon reflection of their discussion in this paper, it is clear that they struggled with the nature of natural groups and the correct placement of the new species. In fact, in this paper, their comparisons among these six southern species inhabiting rivers of the Tampico Embayment drainage suggested that they could constitute a natural grouping and were considered "provisionally" in the genus *Dionda* (Hubbs and Miller, 1977). Later on, through studies using morphological characters from a large number of North American cyprinid genera, *Dionda* (based on two different species) was considered a genus separate from *Hybognathus* and closely related to *Campostoma* and *Nocomis* (Mayden, 1989).

Few phylogenetic hypotheses for species from this genus are available. In those conducted to date, the genus has always been considered as monophyletic based on morphological characters (Coburn and Cavender, 1992; Mayden, 1989). However, comprehensive phylogenetic relationships among all recognized species of the genus *Dionda* were not examined until the allozyme electrophoresis studies by Mayden et al. (1992) using 32 gene loci. These authors unambiguously resolved *Dionda* as a monophyletic group, sister group to *Hybognathus* and closely related to the genus *Campostoma*. Within *Dionda*, they recovered the *D. episcopa* complex as a clade, comprising nine distinct and diagnosable species, closely related to an undescribed species of *Dionda* from Pánuco drainage in Mexico. On the contrary, the southern species of *Dionda* that inhabit the Tampico Embayment in East Central Mexico, did not form a monophyletic group in this allozyme analyses (Fig. 2A).

Recently, Schönhuth et al. (2007) analyzed relationships among Mexican cyprinids using cytochrome *b* gene sequence data and resolved *Dionda* as a non-monophyletic grouping for the first time when other related genera were included in the phylogeny (Fig. 2B). The relationships between species of the genus *Dionda* were also different than those proposed in the prior allozyme analyses, and four species (*D. mandibularis*, *D. melanops*, *D. argentosa* and *D.* sp. from the Colorado and Guadalupe rivers) were not included in the mitochondrial analyses.

Thus, different phylogenetic hypotheses currently exist for *Dionda* depending of the use of nuclear (allozymes) or mitochondrial (cyt*b*) character data. Most gene trees analyses assume that the gene tree within the nominal species is monophyletic for the genes under study. However, certain alleles in one species may appear more closely related to alleles from different species than to conspecific alleles and may lead to erroneous evolutionary interpretations in closely related taxa (Funk and Omland, 2003). This assumption requires that the nominal species being studied

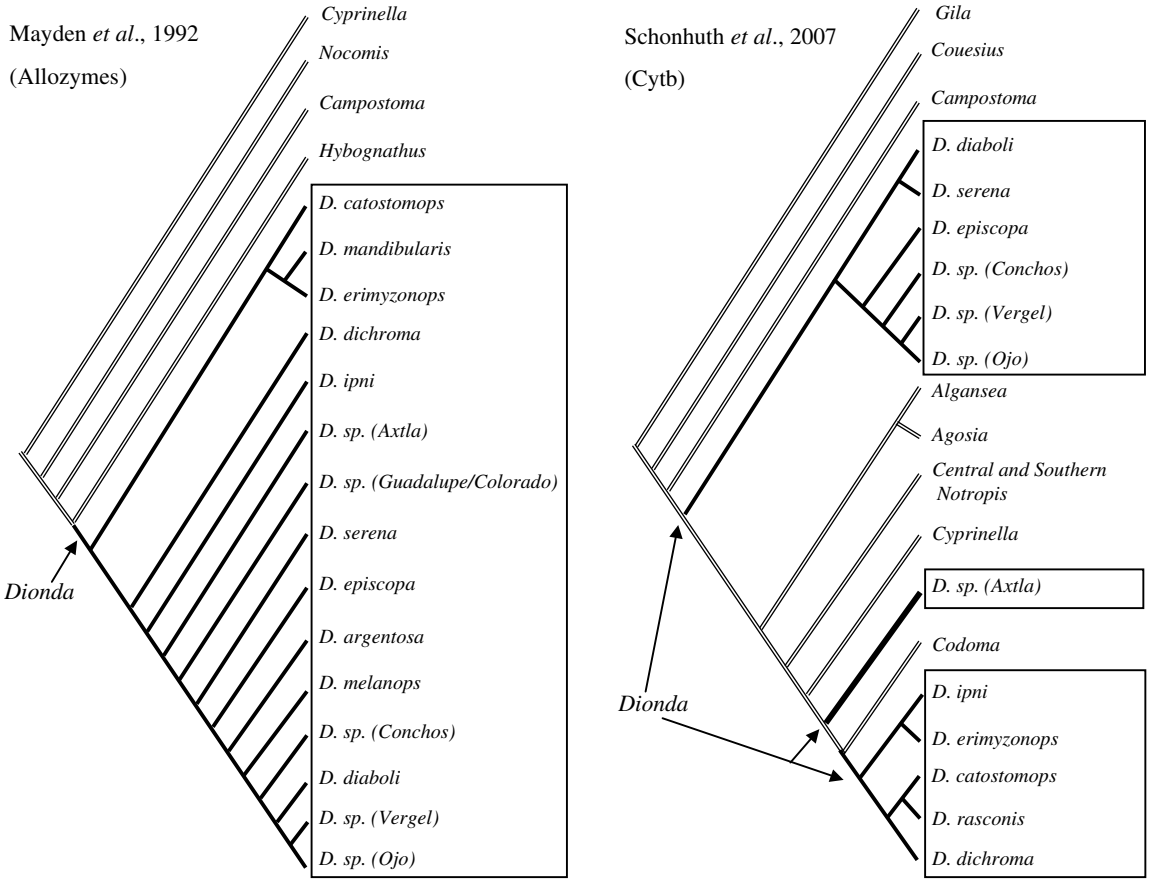

Fig. 2. Previous relationships suggested among the cyprinid taxa analyzed based on (A): allozyme analyses and (B): cyt*b* sequence analyses.

represent genetically and reproductively independent lineages and that lineage coalescence occurred rather rapidly with the divergence of the species and retained primitive alleles or haplotypes were lost. To avoid this potential type of error in phylogeny reconstruction some authors have argued that the repeatability of clades in a phylogeny recovered from separate gene analyses should be the primary criterion to establish the reliability of clades, rather than bootstrap support from a single combined data matrix (Chen et al., 2003).

In this study, we selected one mitochondrial (cytochrome *b*) and three unlinked nuclear genes (Rhodopsin, intron S7 and Recombination Activating Gene 1) with different rates of molecular evolution. We used these genes to reconstruct the phylogenetic relationships among 15 out of 16 recognized and undescribed species of *Dionda* covering their entire distribution. We then used these phylogenetic analyses to examine the evolutionary history of the group and to revise the classification of the genus. Our taxonomic and character sampling allows us to address several different issues: (1) test for intra- and interspecific relationships through analyses of different and geographically distant individuals of each species included in the widely distributed genus *Dionda*; (2) test for monophyly of the genus through the inclusion of all recognized and undescribed species of the genus and other proposed closely related genera based in previous morphological and molecular analyses (Mayden, 1989; Coburn and Cavender, 1992; Mayden et al., 1992; Schönhuth et al., 2007); and (3) resolve relationships among nine putative closely related genera including all genera of the family Cyprinidae in Mexico. The inference of a molecular phylogeny by the comparison of nuclear and mitochondrial gene sequence data also aids in the examination of the evolutionary history of those cyprinid fishes that inhabit this important transition area, from the Nearctic to Neotropical freshwater fish faunas in North America.

2. Materials and methods

Fifty specimens of *Dionda* were collected by electrofishing and seining throughout the range of the genus (from coastal Nautla River 20°N in Veracruz, to Pecos River 34°N in New Mexico). These specimens represent 11 putative species and 4 undescribed species previously recognized by allozyme analyses (Mayden et al., 1992) and morphological comparisons (Mayden and Miller, unpub.). We include all species currently in the genus *Dionda* except *D. rasconis*. Multiple collections at known localities of *D. rasconis* produced no individuals of this species, which was also not available in the study by Mayden et al. (1992). This species is of significant conservation concern, and it may be extinct. The analyses also included 34 species from nine putative related genera (*Agosia*, *Algansea*, *Campostoma*, *Codoma*, *Cyprinella*, *Notropis*, *Yuriria*, *Nocomis* and *Hybognathus*) based on previous molecular phylogeny (Schönhuth et al., 2007), and morphology and allozyme data (Hubbs and Miller, 1977; Mayden, 1989; Mayden et al., 1992).

A list of specimens examined is provided in Appendix A. Voucher materials are deposited in ichthyological collections at the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); the Universidad Michoacana de San Nicolás de Hidalgo, Michoacana, Mexico (CPUM); Saint Louis University, St. Louis, Missouri, USA (SLU); and The University of Alabama Ichthyological Collection, Tuscaloosa, Alabama, USA (UAIC).

Four molecular regions were selected for the study: the complete mitochondrial cytochrome *b* gene (cyt*b*) and three nuclear genes: S7 ribosomal protein (S7, approx. 880 bp containing the first intron), recombination activating gene 1 (Rag1, approx. 1500 bp) and Rhodopsin (Rhod, approx. 850 bp). DNA extraction from tissue samples was performed using QIAamp Tissue Kits (QIAGEN Inc., Valencia, CA, USA), amplification and primers for cyt*b* gene were detailed in Schönhuth and Doadrio (2003). Primers used for the three nuclear genes have been published by Chow and Hazama (1998) for S7, by Chen et al. (2003) for Rhod and by Lopez et al. (2004) for Rag1. All PCR amplifications were conducted in 50 μl reactions. When more than one band occurred in the S7 nuclear gene PCRs, DNA were purified using DNA Gel extraction (QIAGEN Inc., Valencia, CA, USA). Primers for direct sequencing of the purified PCR were the same as those used for the PCR amplification. Purified PCR products were sent to Macrogen for sequencing. Sequences specifically obtained for this study have been deposited in GenBank under Accession Nos. EU082467–EU082753.

cyt*b* sequences obtained in this study were combined with previously published sequences for Mexican *Notropis* (Schönhuth and Doadrio, 2003: AF469130-1, AF469133, AF469135–6, AF469158–9, AF469153–4, AF169137, AF469140–1, AF469160, AF469163) and *Dionda*, *Campostoma* and *Algansea* (Schönhuth et al., 2007: DG324062–DG324103). From the 111 taxa sequenced for cyt*b* analyses, only those representative specimens from each mitochondrial clade were selected to sequence the three nuclear genes (76 taxa). Sequences of each gene were aligned manually with outgroup species from *Couesius plumbeus* and *Gila pandora*. No ambiguous alignments or gaps were found in cyt*b*, Rag1 and Rhod. Nuclear S7 sequences were aligned using Clustal X ver1.85 (Tomson et al., 1997) and corrected by eye. Multiple indels were detected. The size of S7 sequences varied for all Cyprinidae examined from 836 to 880 bp. Maximum aligned sequence lengths were 1141 bp (cyt*b*), 841 bp (Rhod), 1512 bp (Rag1), and 993 bp with indels (S7). No characters were excluded for the analyses. Sequences were analyzed in five different data sets (one for each DNA region and one for the combined data). cyt*b* was chosen to have the most complete representation among species and the remaining genes were sequenced in a smaller set of taxa. Observed genetic divergences mentioned herein are based on cyt*b* uncorrected *p*-distances.

In the combined data set four genes were concatenated for each taxon in a total of 4487 bp.

Phylogenetic trees were estimated for each data set (each gene separately and concatenated sequences) using Maximum Parsimony (MP), Minimum Evolution (ME) and Bayesian Inference (BI). MP analyses involved heuristic searches with 10 random stepwise additions replicates, MULTREE option and tree bisection-reconnection (TBR) branch swapping. All transformations were weighted equally. The hierarchical likelihood ratio test (hLRT) implemented in MODELTEST v3.4 (Posada and Crandall, 1998) was used to find the evolutionary model that best fit five different sequence data set (each gene separately and the combined data set), and optimized parameter values were used to estimate ML distances for ME analyses. Models and parameters that were selected under hLRT are summarized in Table 1. For the separate gene data sets, different BI analyses were conducted: BI_cyt*b* with three partitions (cyt*b* 1st + 2nd + 3rd codon position); BI_Rag1 with three partitions (Rag1 1st + 2nd + 3rd codon position); BI_Rhod with three partitions (Rhod 1st + 2nd + 3rd codon positions) and BI_S7 with no partitions; for the combined data set BI analyses were performed with ten partitions BI_10all (cyt*b* 1st + 2nd + 3rd codon positions + RAG1 1st + 2nd + 3rd codon positions + Rhod 1st + 2nd + 3rd codon positions + S7). Model parameters were estimated independently for each of the respective data partitions using the unlink command in Mr. Bayes v3.03.

Robustness of the inferred trees was evaluated using bootstrap analysis (Felsenstein, 1985) on 1000 pseudoreplications for MP and ME. For BI 1,000,000 generations were implemented, sampling the Markov chain at intervals of 100 generations. A total of 1000 out of 10,000 resulting trees were discarded as "burn-in". Bayesian inferences were performed twice beginning with different starting trees. Support for tree nodes was determined based on values of Bayesian posterior probabilities obtained from a majority-rule consensus tree. Phylogenetic analyses were conducted with PAUP* v4.0b10 (Swofford, 2001) and Mr. Bayes v3.03 (Huelsenbeck and Ronquist, 2001).

3. Results

3.1. Sequence analyses

Complete cyt*b* sequences were analyzed for 111 specimens representing 50 species of 10 putative genera (*Agosia*, *Algansea*, *Campostoma*, *Codoma*, *Cyprinella*, *Dionda*, *Hybognathus*, *Nocomis*, *Notropis* and *Yuriria*) from 110 different localities. The phylogeny estimated from the full set of sequences was restricted of those taxa represented by the four gene regions. The complete 4 gene sequences were analyzed for 76 specimens from 76 localities, including *Couesius plumbeus* and *Gila pandora* as outgroup taxa.

Plots of transitions and transversions against uncorrected genetic distance indicated an absence of nucleotide saturation in cyt*b*, Rhod, Rag1 and S7. Of the 1141 bp aligned for cyt*b*, 535 sites were variable and 469 (17.9% 1st position; 3.0% 2nd; 79.1% 3rd) were parsimony informative. Of the 993 bp aligned for S7, 46.72% were parsimony informative sites, 11.05% of the 841 bp aligned for Rhod and 13.95% of the 1512 bp aligned for Rag1 (Table 1).

The most complex evolutionary model is preferred for cyt*b*, whereas simpler models were more appropriate for S7 and Rag1 that do not have invariant sites (Table 1). S7 stands apart from the others in its much more uniform

Table 1
Summary of molecular characterization and model parameters obtained of cyprinids species analysed for the inferred phylogenies

Gene region	mtDNA		nDNA		Combined data set (10 partitions)
	cyt*b*	S7	Rhod	Rag1	
Individuals analyzed	111	76	77	76	75
Number base pairs in alignment	1141	993	841	1512	4487
Variable sites	535	623	128	287	—
Parsimony informative sites	469 (41.10%)	464 (46.72%)	93 (11.05%)	211 (13.95%)	—
A	0.25350	0.30698	0.17729	0.25697	—
C	0.28785	0.14676	0.34095	0.24049	—
G	0.16794	0.19600	0.24480	0.27019	—
T	0.29071	0.35026	0.23695	0.23236	—
Selected model (hLRT) all positions	(GTR+I+G)	(HKY+G)	(HKY+I+G)	(K80+G)	—
Gamma shape (Γ)	0.9865	2.2923	1.3956	0.1116	—
Prop. Invar. sites (I)	0.5018	0	0.7261	0	—
Selected model (hLRT) 1st codon position	(TrN+I+G)	—	(JC+I+G)	(F81+I)	Partitions
Selected model (hLRT) 2nd codon position	(HKY+G)	—	(F81)	(HKY+I+G)	Partitions
Selected model (hLRT) 3rd codon position	(TrN+I+G)	—	(TVM+G)	(HKY+G)	Partitions
Node resolution*	6/1/1 (ME)	6/0/2 (ME)	4/0/4 (ME)	6/1/1 (ME)	7/0/1 (ME)
	7/0/1 (MP)	5/1/2 (MP)	3/2/3 (MP)	8/0/0 (MP)	8/0/0 (MP)
	8/0/0 (BI)	5/1/2 (BI)	3/3/2 (BI)	6/2/0 (BI)	8/0/0 (BI)

nDNA, nuclear DNA; mtDNA, mitochondrial DNA. (*): number of high (⩾70% ME. MP; ⩾95% BI)/moderate (<70–50% ME. MP; <95% BI)/low (<50% ME. MP. BI) for the eight clades.

pattern of among-site rate variation. In addition, this gene presented high number of insertions and deletions. The alignment of S7 sequences required the insertion of 50 indels ranging in size from one to 20 bp. The compared nucleotide composition of the 4 genes indicates a high frequency of C in Rhod and lower frequency of this base in S7 (Table 1). Guanine was found at particularly low frequency in third codon positions (G: 0.1119) in cyt*b*, as also occurs in *Notropis* (Bielawski and Gold, 1996; Schönhuth and Doadrio, 2003). The S7 sequences showed a moderate bias towards A and T, as occurred in previously reported AT-rich intron sequences in fishes (Orti et al., 1996; Lavoué et al., 2003). However, the null hypothesis of homogeneity of frequency among bases across taxa was not rejected.

The range of sequence divergences (uncorrected *p*-distances) found in cyt*b* among species pairs from each of the putative genera were as follows: *Algansea*: 2.1–8.6%; *Agosia* 9.4%; *Hybognathus*: 9.3%; *Codoma*: 2.4–9.4%; *Campostoma*: 2.9–11.4%; central and southern *Notropis* clade: 4.5–14.3%; *Cyprinella*: 1.4–17.2%; and *Dionda*: 1.3–20.6%. Ranges in divergence for each gene between major clades are shown in Table 2.

3.2. Phylogenetic analyses

All separate analyses of mitochondrial and nuclear data sets revealed the genus *Dionda* as a non-monoplyletic group (Figs. 3 and 4). Mitochondrial analyses of 111 taxa recovered phylogenies showing eight major clades. These clades are herein referred to as: (A) the southern "*Dionda*" clade, (B) *Codoma* clade, (C) *Cyprinella* clade, (D) *Hybognathus* clade, (E) central and southern *Notropis* clade, (F) *Algansea* + *Agosia* clade, (G) Northern *Dionda* clade, and (H) *Campostoma* + *Nocomis* clade (Fig. 3). Although some topological incongruities exist between trees recovered from nuclear genes, most of the intergenic nodes (common to separate data set analyses) correspond to the eight major clades recovered in cyt*b* analyses, and were generally well-supported in the nuclear genes with some exceptions (Tables 1 and 2). However, there are some topological incongruences between gene trees: The genus *Cyprinella* was always a well-supported clade, except in the analyses of S7, which suggested *Cyprinella* was not monophyletic. The *Hybognathus* clade is monophyletic in the analyses of cyt*b* and Rag1, but it is not in the S7 and Rhod analyses. However the non-monophyletic genus *Hybognathus* was poorly supported. *Campostoma* is always the sister clade of *Nocomis* except in the Rhod analyses. Thus, the topological conflicts are limited to two nodes: *Cyprinella* is a non-monophyletic group in the S7 analyses, and *Nocomis* + *Campostoma* are not sister groups in the Rhod analyses.

3.3. Genus Dionda

In stark contrast with previous phylogenetic studies involving the genus *Dionda*, the 15 species included in the current analysis did not form a monophyletic group. Our phylogenetic analyses strongly support the separation of *Dionda* into two well-differentiated lineages (see Figs. 3–5: clades A and G) that have disjunct geographic distributions and no immediate common ancestor. One of these clades (A) includes southern species that inhabit rivers mainly of the Tampico Embayment drainage of Mexico. This clade forms the sister group to genus *Codoma*, and is closely related to *Cyprinella* clade. The other highly divergent *Dionda* clade (see Figs. 3–5: clade G) includes northern species of the *D. episcopa* complex that inhabit mainly the Rio Grande region. This clade (G) is recovered as the sister

Table 2
Comparison of estimated support considering single gene and concatenated sequences for the major nodes in the phylogeny for the three methods of analyses

	Major clades	Single gene support (ME/MP/BI)/genetic divergence*								Concatenated support
		cyt*b*		S7		Rhod		Rag1		
A	*Tampichthys*	<50/<50/95	8.5–15.5	79/65/75	1.8–7.7	90/91/100	0.2–1.4	97/93/100	0.1–1.3	99/98/100
B	*Codoma*	100/100/100	2.4–9.4	100/100/100	0.2–2.0	75/60/96	0.1–0.5	96/96/100	0.06–0.7	100/100/100
C	*Cyprinella*	95/80/100	1.4–17.2	—/—/—	0.3—9.7	88/75/58	0.2–2.3	100/98/100	0.06–2.2	100/99/100
D	*Hybognathus*	99/79/100	9.3	—/—/—	8.8	—/—/—	1.1	98/100/100	1.6	100/100/100
E	*Notropis* + *Yuriria* clade	96/73/100	4.5–14.37	100/100/100	0.8–5.2	<50/64/74	0.3–1.4	<50/72/55	0–0.8	100/100/100
F	*Algansea* + *Agosia* clade	100/96/100	(2.1–8.6)13.9	100/100/100	(0.6–1.7)4.3	—/<50/61	(0.1–0.5)1.6	100/100/100	(0.1–0.4) 0.8	100/100/100
G	*Dionda*	99/94/100	1.3–15.9	100/100/100	0.7–5.9	98/100/100	0.1–1.1	100/100/100	0.06–1.3	100/100/100
H	*Campostoma* + *Nocomis* clade	57/77/93	(2.9–11.4)17.4	73/72/99	(1.1–6.9)11.8	—/—/—	(0.1–0.8)2.4	54/78/71	(0.2–1.2) 2.5	—/90/100
	Tampichthys – *Dionda*	—	16.3–20.6	—	14.4–19.2	—	4,5–5.6	—	4.2–5.0	—

ME, minimum evolution; MP, maximum parsimony; BI, Bayesian inference. (*), range of uncorrected *p* distances percentage; (—), monophyly not supported.

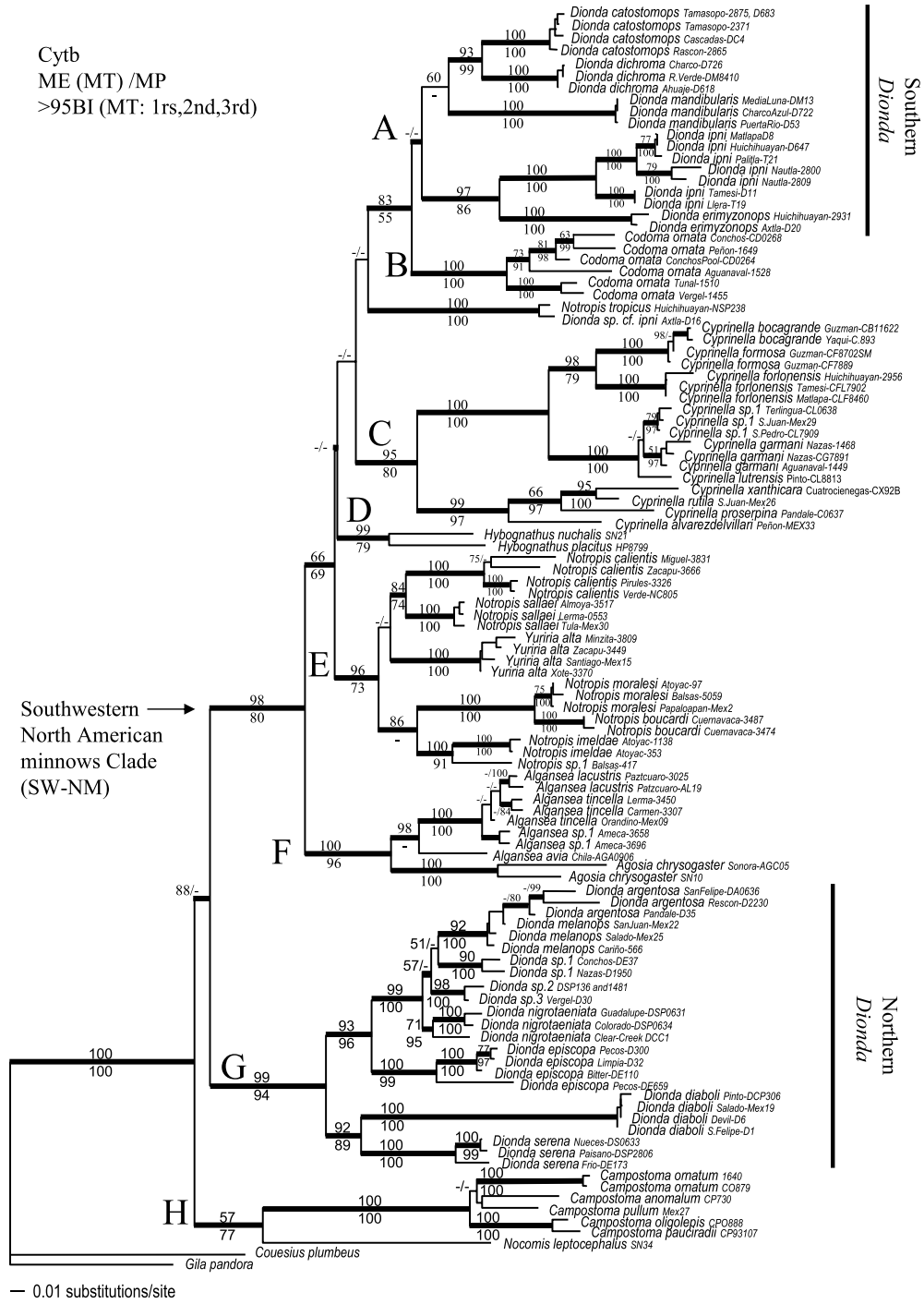

Fig. 3. Minimum evolution tree representing phylogenetic relationships among 111 cyprinids taxa analyzed based on cyt*b*. The branch lengths are proportional to inferred distances. Numbers on branches indicate bootstrap support for ME and MP (based on 1000 replications) and bold branches indicate posterior probabilities >95 for BI (based on 1,000,000 generations). Collection numbers and drainages are given behind species names. Major lineages referred in the text are also designated by letters.

group to large clade of southwestern North American minnows.

This large group of southwestern North American minnows (see Figs. 3–5: SW–NM) is a well-differentiated and well-supported clade in all separate gene analyses and included species from seven putative different genera (*Codoma*, *Cyprinella*, central and southern Mexican *Notropis* (including *Yuriria*), *Hybognathus*, *Agosia*, *Algansea*) plus the southern "*Dionda*" clade. Besides *Dionda*, all other genera were consistently resolved as monoplyletic in all gene analyses with the exception of *Cyprinella* in S7 analyses and *Hybognathus* in S7 and Rhod analyses. The SW–NM clade includes six major clades (A, B, C, D, E and F) that were recovered in most of the analyses (Figs. 3–5).

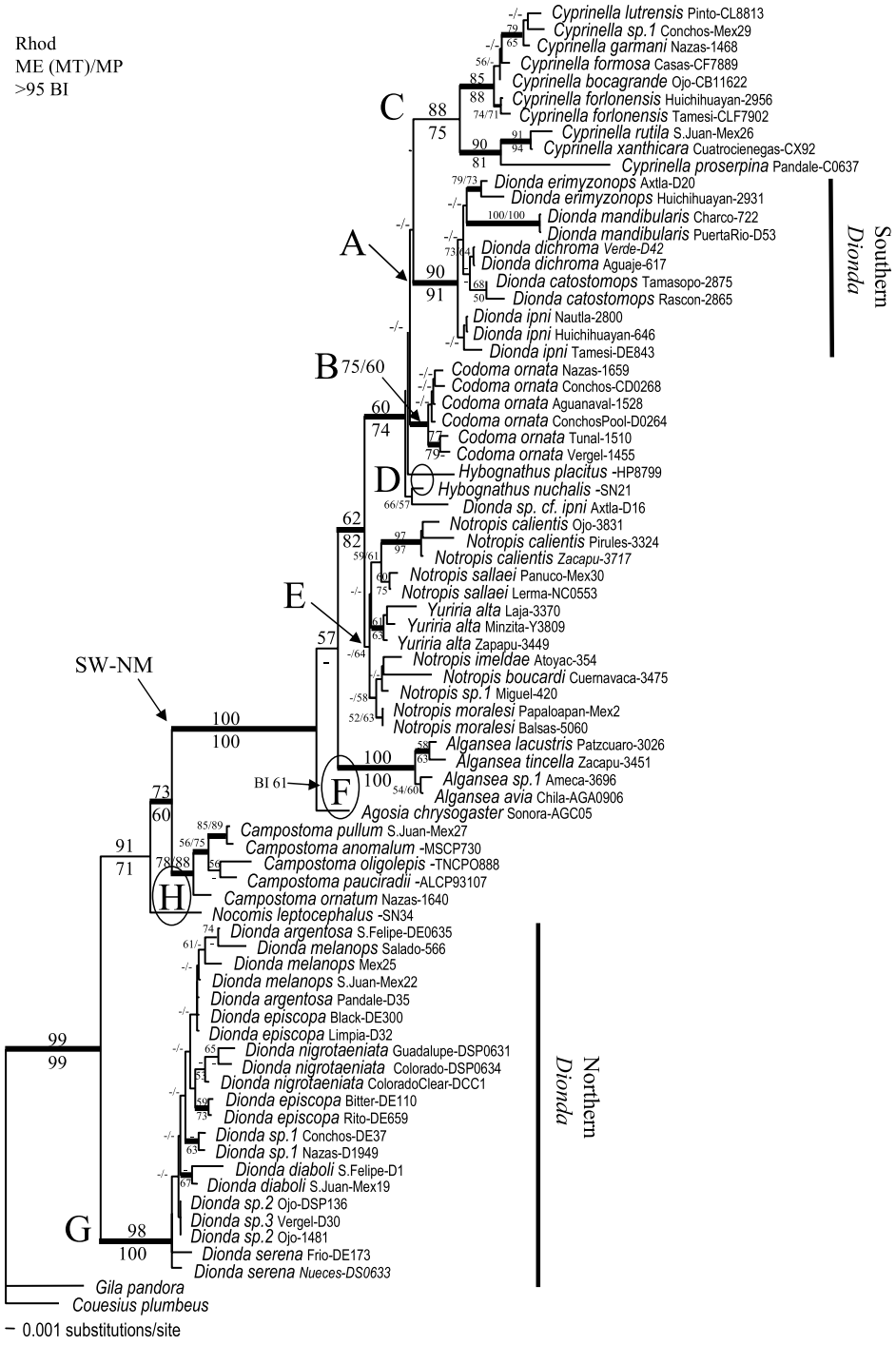

Fig. 4. Minimum evolution tree showing phylogenetic relationships among 76 representative cyprinids taxa analyzed based on nuclear data sets: (A) Rhod; (B) Rag1; (C) S7 sequences; numbers on branches indicate bootstrap support for ME and MP, respectively, and bold branches indicated posterior probabilities >95 for BI. Collection numbers and drainages are given behind species names. Major lineages referred in the text are also designated by letters.

The genus *Dionda* was previously recognized as a monophyletic group based on morphology and allozymes, and hypothesized to be closely related to *Campostoma*, *Hybognathus* and *Nocomis* (Mayden, 1989; Coburn and Cavender, 1992; Mayden et al., 1992). Our phylogenetic analyses based on nuclear and mtDNA sequence data consistently refute this hypothesis. Instead, our analyses strongly support species currently assigned to the genus *Dionda* that inhabit rivers of the Tampico Embayment (in central-east Mexico) as constitute a natural grouping as previously suggested by Hubbs and Miller (1977), but immersed in the large SW–

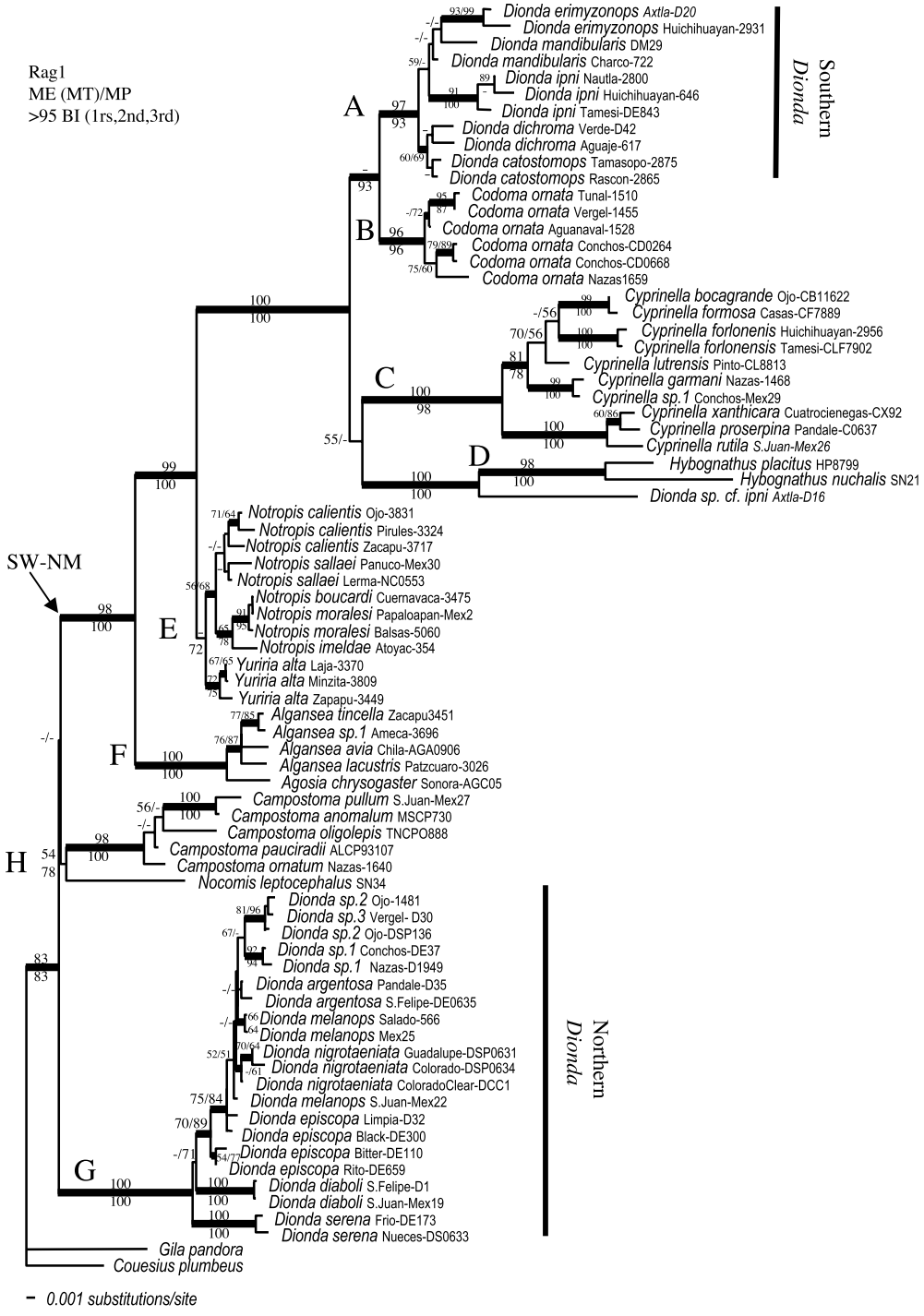

Fig. 4 (*continued*)

NM clade. However, this southern "*Dionda*" clade is more closely related to the genus *Codoma* and to *Cyprinella* within the SW–NM clade, than to species of the genus *Hybognathus*. Southern species of genus *Dionda* are highly divergent from the northern lineage of *Dionda*, which are recovered with strong support and basal to the SW–NM clade in all separate gene analyses. The genus *Campostoma* is a well-supported monophyletic clade, sister to *Nocomis*, in all analyses except with Rhod.

3.4. Northern species of the genus Dionda

Nine species of *Dionda* formed a well-supported monophyletic group recovered in all separate gene analyses. This clade is also unique in possessing three derived deletions and one derived insertion in the S7 alignment data set. The Northern *Dionda* clade was recovered as the sister group of the large SW–NM clade in all but the Rhod analyses, where it was recovered in a basal position of the phylogeny. This clade includes all species assigned to the genus

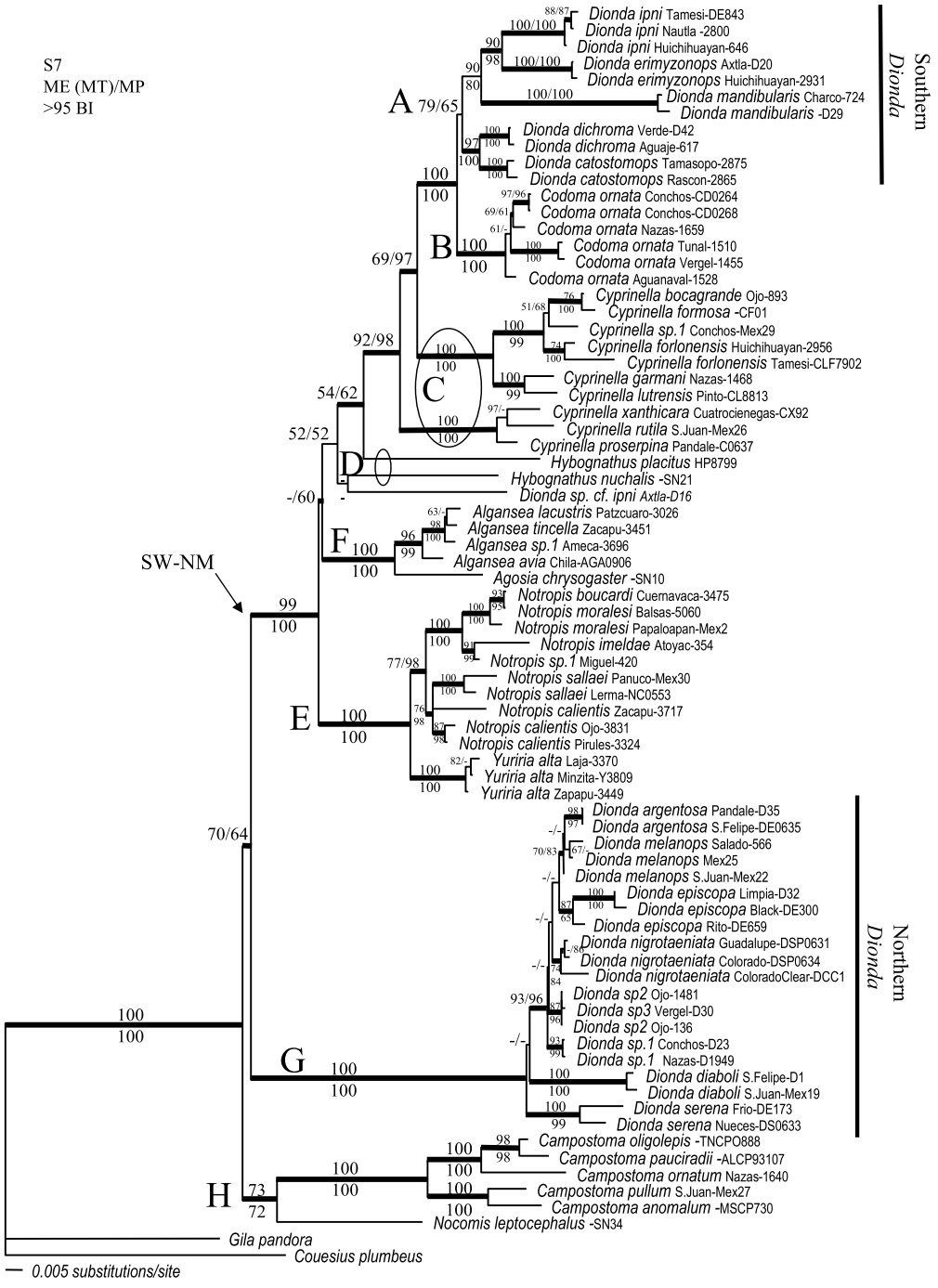

Fig. 4 (*continued*)

Dionda inhabiting different drainages over the northern distribution range of the genus. Six of the nine species (*D. episcopa*, *D. serena*, *D. diaboli*, *D. argentosa*, *D. nigrotaeniata* and *D. melanops*) are described taxa, whereas the other three are undescribed species based on previous allozyme evidence (Mayden et al., 1992). These three species, which inhabit the upper Mezquital and Rio Grande in Mexico are referable to *D.* sp.1 (Río Conchos), *D.* sp.2 (Ojo de Agua) and *D.* sp.3 (El Vergel Spring) and are currently being described by Mayden. Here we followed Gilbert (1998), who realized that *D. nigrotaeniata* was a name available for one assumed undescribed species inhabiting the Colorado and Guadalupe drainages in Texas. Miller et al. (2005) used the name *D. couchi* rather than *D. melanops*. Both names were published simultaneously. Mayden et al. (1992), as first revisers of the genus, selected *D. melanops* as the appropriate name for this taxon and *D. couchi* is thus a junior subjective synonym.

These nine species inhabit different tributaries of the Rio Grande drainage in northern Mexico and the southwestern

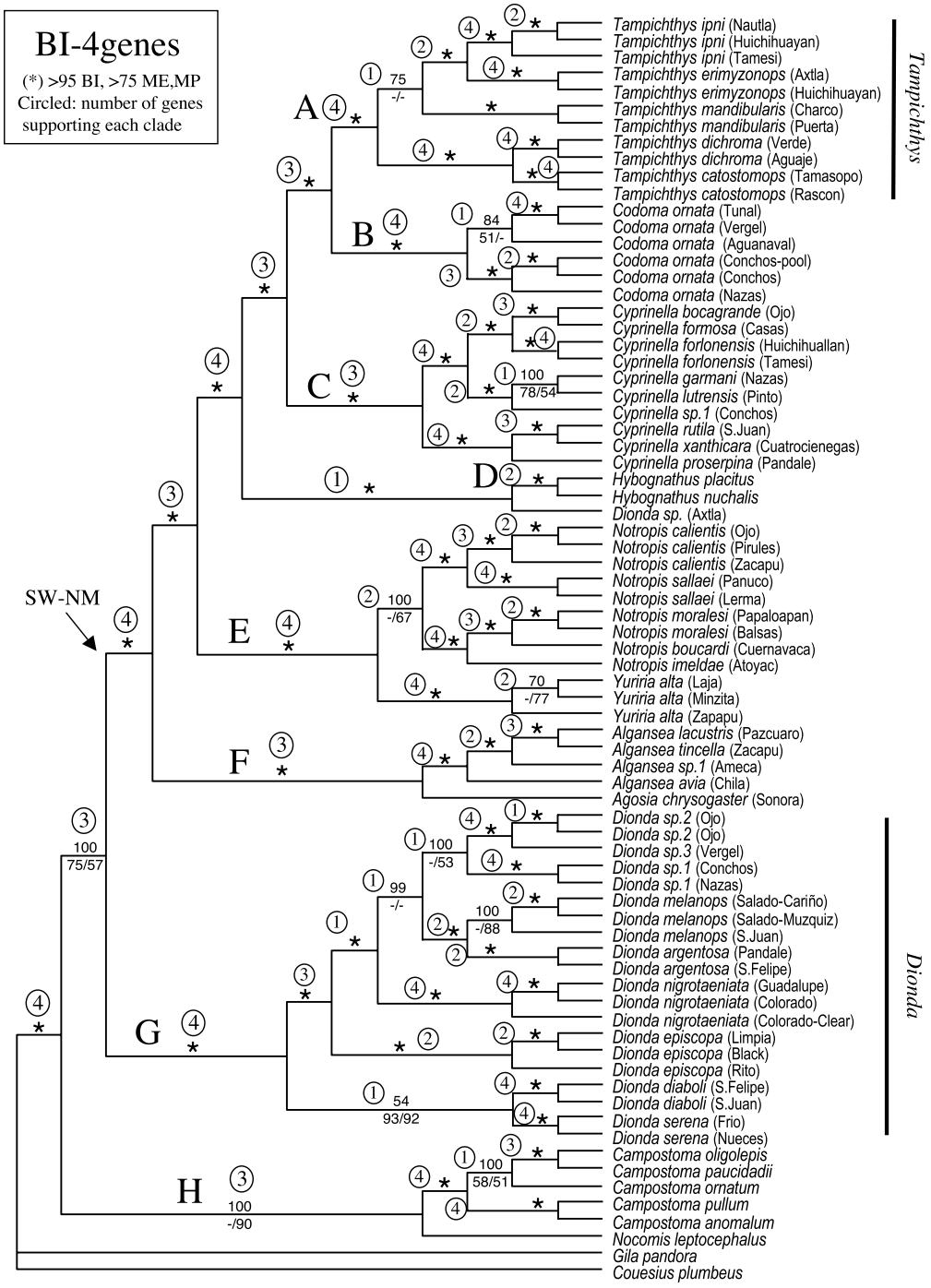

Fig. 5. Reconstructed phylogeny of the cyprinids analyzed using a Bayesian phylogenetic approach with the combined data set (one mitochondrial and three nuclear genes). Numbers on branches indicate posterior probabilities for the BI, and bootstrap support for ME and MP, respectively. * indicates >95 posterior probability support for the BI analyses and >75 bootstrap support for ME and MP. Circled numbers 1–4 indicate the number of genes that supported each clade in separate gene analyses.

United States, reaching upper waters of three drainages of the Western Gulf Slope (Nueces/Frio, Guadalupe/San Antonio and Colorado) in the United States, and south as far as Río Tunal (upper Mezquital, north-central Mexico, Pacific slope) and the endorheic Río Nazas drainage (Durango, Mexico) (Fig. 6). Divergences within this group ranged from 1.3% (*D.* sp. Vergel–*D.* sp. Ojo de Agua) to 15.9% (*D. diaboli–D. argentosa*).

All separate gene analyses supported *D. diaboli* and *D. serena* as the most divergent species within northern *Dionda* clade, while the remaining seven species always formed a well-supported clade. *Dionda diaboli* specimens sampled in four different tributaries of Rio Grande were always recovered in a well-supported clade with scarce divergences among specimens (0.2–1.0%). *Dionda serena* inhabits headwaters of the Nueces/Frio drainage, Texas,

Fig. 6. Current distribution range of both genera (*Dionda* and *Tampichthys*) is represented in grey patterns. Trans-Mexican Volcanic Belt, main drainages and putative river captures mentioned in the text are also indicated.

and displayed high divergences (up to 4.4%) between specimens from the Nueces and Frio rivers. cyt*b* genetic divergences among *Dionda episcopa* specimens range from 0.2% to 7.6%, and are highest among those specimens from the upper and lower portions of the Pecos River. Although the cyt*b* and S7 analyses recovered these *D. episcopa* specimens as a well-supported clade, in Rhod and Rag1 analyses their monophyly was not supported. *Dionda melanops* and *D. argentosa* were closely related species in all analyses with divergences between both species ranging from 2.6% to 4.7%. The two undescribed species inhabiting the upper Mezquital drainage (*D.* sp.2 and *D.* sp.3) were sister species in all analyses and they displayed low divergence (1.3%). Specimens assigned to *Dionda* sp.1 from the Río Conchos (Rio Grande tributary) and those collected from the Río Nazas drainage (endorheic drainage) showed low levels of divergence (0.4%). All analyses supported the close relationship between these two populations from this undescribed species even though they occur in two independent drainages in northern Mexico (Río Nazas and Río Conchos) that have been separated from one another for considerable time (Minckley et al., 1986). *Dionda nigrotaeniata* from two independent drainages of the Western Gulf Slope (Colorado and Guadalupe rivers) were always in a well-supported clade with divergences ranging from 2.1% to 5.5%, with those specimens from Clear Creek (Colorado River Drainage) the most divergent of the species.

The highest intraspecific divergences observed in the *Dionda episcopa* complex were found among specimens of *D. episcopa* from the upper and lower Pecos River (7.2–7.6%), between *D. serena* from the Nueces and Frio rivers

(4.2–4.4%), and between specimens assigned to *D. nigrotaeniata* from the Colorado and Guadalupe drainages (5.1–5.5%). These intraspecific genetic divergences were higher than those found among three non-barbeled species of genus *Algansea* (2.1–4.0%). However, they are similar to intraspecific divergences found between specimens of *D. ipni* (4.7–7.3%), and to those existing between specimens of *Notropis calientis* (5.8–6.9%). These high intraspecific divergences suggest ancient differentiation among populations of these species, and further analyses are required to fully determine their taxonomic status.

3.5. Southern Dionda clade and related genera

Five recognized species included in genus *Dionda* inhabiting rivers of the Tampico Embayment drainage formed a highly divergent clade separate from the northern *Dionda* clade. This heterogeneous clade includes *D. ipni*, *D. catostomops*, *D. dichroma*, *D. mandibularis* and *D. erimyzonops*. Divergences within this group ranged from 8.5% (*D. dichroma–D. catostomops*) to 15.5% (*D. mandibularis–D. ipni*), and rose to 15.7% when an undescribed species of the Río Pánuco drainage (previously named as *Dionda* sp. *cf. ipni* from Río Axtla in Mayden et al., 1992) was considered. This species from Pánuco basin was never recovered within either of the two *Dionda* clades (A or G), but was always included in the large SW–NM clade. Outside of *Dionda*, the phylogenetic position of this species was not resolved in mitochondrial analyses but nuclear analyses support this species as closely related to the genus *Hybognathus*. S7 and Rhod analyses recovered it as sister to *H. nuchalis* and Rag1 analyses supported it as sister species to the clade (*H. nuchalis* + *H. placitus*).

Dionda ipni is the only species of this clade known to also inhabit rivers from the Mexican Coastal Plain outside the Pánuco–Tamesí drainage. This species seems to have a more generalized habitat than *D. erimyzonops* with which it shares a large degree of sympatry. Within *D. ipni* there was high intraspecific differentiation (excluding *D.* sp. *cf. ipni* from Río Axtla) observed across the different rivers sampled (Pánuco–Tamesí system and Río Nautla) (4.7–7.2%). All specimens assigned to *D. ipni* were recovered as the sister group to *D. erimyzonops* in mitochondrial and S7 analyses, but not with the other genes. *Dionda catostomops* was consistently recovered as the sister species to *D. dichroma* in all the analyses. The position of *D. mandibularis* within this clade is unclear, and its relationships varied in the separate gene analyses.

All species of the southern "*Dionda*" clade, except the undescribed species from the Río Axtla, were recovered as the sister group to the genus *Codoma*. Similar genetic divergences were found between *Codoma* and *Cyprinella* (14.4–17.7%) and between *Codoma* and southern "*Dionda*" species (12.6–17.1%). The divergences between the southern species of the genus "*Dionda*" and the undescribed species from the Río Axtla range between 13.7% and 15.7%. These values are higher than divergences found between this undescribed species and *Hybognathus* (12.7%). *Dionda* sp. (Río Axtla) was recognized on the basis of allozyme data, and despite being morphologically similar to *D. ipni*, it displayed fixed differences from *D. ipni* at 14 loci and was recovered as the sister group to *D. episcopa* complex clade (Mayden et al., 1992). This previous allozyme analysis recovered the *D. ipni* complex (including this undescribed species of *Dionda* from the Pánuco drainage) as paraphyletic, because this species was more closely related to species of the *Dionda episcopa* complex than to the remaining species of *Dionda* from the Pánuco–Tamesí drainages in central Mexico (Mayden et al., 1992).

Genetic divergences of cyt*b* show that *D.* sp. Río Axtla was more closely related to the southern "*Dionda*" clade (13.7–15.7%) than to the Northern *Dionda* clade (16.3–18.2%), but the relationship of this undescribed species within the SW–NM clade remains unclear. cyt*b* analyses recovered this species as either sister species to the southern "*Dionda*" + *Codoma* clade or sister species to the ((southern "*Dionda*" + *Codoma*) + *Cyprinella*) group. However, nuclear gene analyses supported this species as more closely related to *Hybognathus*. This highly divergent form previously identified in Mayden et al. (1992) as *Dionda* sp. Río Axtla is currently being described by Mayden.

All specimens of the genus *Codoma* examined were recovered in a well-supported clade (B) in all gene analyses, grouping specimens from different drainages (Mezquital, Nazas, Aguanaval, Rio Grande) as the sister group of the southern "*Dionda*" clade in most of the analyses (except Rhod). *Codoma* is currently a monotypic genus, with only *C. ornata* recognized. This species is widely distributed in Mexico, occurring in five north-western drainages. Genetic divergences found among specimens from four different drainages in Mexico range from 2.4% to 9.4% in cyt*b*. This range is similar to those divergences found between recognized species of the eight major clades recovered in this study. These high mitochondrial divergences among specimens of *Codoma* are exhibited in the nuclear genes analyzed (Table 2), suggesting that the species currently known as *C. ornata* actually consist of a complex of several species.

In all analyses species of the genus *Cyprinella* included in our study were part of the SW–NM group, and were always resolved as a clade closely related to *Codoma* and the southern *Dionda* clade. The 10 species of the genus *Cyprinella* included in cyt*b* analyses were recovered as a monophyletic group (C) and presented the highest intragenera genetic divergence ranging from 1.4% (*C. bocagrande–C. formosa*) to 17.2% (*C. xanthicara–C. garmani*). Interestingly, nuclear regions also showed high intra-genera divergences (Table 2). All gene analyses recovered two highly divergent and well-supported clades for species of *Cyprinella*: clade C1 was comprised of four species: *C. rutila*, *C. xanthicara*, *C. proserpina* and *C. alvarezdelvillari* (only partial cyt*b* from the latter species); and clade C2 was comprised by six species: *C. bocagrande*, *C. formosa*, *C. forlonensis*, *C.* sp.1, *C. lutrensis* and *C. garmani*. These

two clades (C1 and C2) were recovered as a monophyletic group in all mitochondrial and nuclear analyses (cyt*b*, Rag1 and Rhod) except for S7, in which this genus appears paraphyletic. In the latter analyses the C1 clade was basal to the clade formed by ((southern *Dionda* + *Codoma*) C2). Comparing S7 aligned sequences for these two clades, C1 presented 3 deletions (of 12, 5 and 1 bp) and 3 insertions (of 4, 3 and 3 bp) with respect to clade C2. However, five out of these six indels in the C1 clade are also present in other major clades, and hence this could be the reason for the apparent paraphyly of *Cyprinella* in the S7 analyses.

Members of the previously identified "Central and Southern Mexican *Notropis* clade" based on cyt*b* analyses (Schönhuth and Doadrio, 2003) include *Notropis moralesi*, *N. boucardi*, *N. calientis* complex, *N. imeldae*, *N. sallaei*, *N.* sp.1 and *N. altus* (=*Yuriria alta*). This monophyletic clade was also recovered in all three nuclear gene analyses. These closely related taxa form clade (E), which is part of the large SW–NM group. Its position within the SW–NM group is always recovered as basal to species from the mitochondrial clades A, B, C and D. Within this clade E, S7 and Rag1 nuclear analyses recovered *N. altus* in a basal group but its position was not resolved in Rhod analyses. In all analyses *N. sallaei* forms the sister species to the *N. calientis* complex. All nuclear analyses support *N. boucardi* and *N. moralesi* as closely related species and sister clade of *N. imeldae* + *N.* sp.1.

Species of *Algansea* were always recovered as a well-supported monophyletic group in all separate gene analyses. Divergences within non-barbeled species were 2.0–4.0%, but increased to 9.0% when the only species analyzed here with barbels (*A. avia*) was included. Relationships within this genus were poorly resolved in Rhod and Rag1 analyses, but S7 and cyt*b* always recovered *A. avia* as sister species of the non-barbeled group (*A. tincella*, *A. lacustris* and *A.* sp.1). All analyses support the hypothesis that specimens currently included in the widely distributed species *Algansea tincella* represent a paraphyletic assemblage and also support the possible existence of different, cryptic species within the *Algansea tincella* complex. *Algansea* was recovered as sister group to the genus *Agosia*, with high support, in all but the Rhod analyses. In the Rhod analyses, the sister close relationship between *Algansea* and *Agosia* is not supported in ME and poorly supported in MP and BI analyses. This clade (F) was always recovered in a basal position within the SW–NM group.

4. Discussion

4.1. Phylogenetic relationships and taxonomy

The phylogenetic analyses presented herein based on both mitochondrial and nuclear gene sequences strongly support the division of the currently recognized genus *Dionda* in two highly divergent clades with disjunct geographic distributions. The four DNA regions analyzed here exhibited different rates of evolution and resolved phylogenetic relationships at different levels. The more variable regions were cyt*b* and S7, and both these genes produced trees with long terminal nodes and short branches with usually weak support for relationships between major clades. However, they also resolved similar relationships among species within the major groups. In contrast, the Rhod and Rag1 genes strongly supported nodes between major clades. However, terminal branches for these two particular nuclear genes were notably shorter than for the other genes, and the nodes within major clades were poorly resolved (particularly in Rhod analyses). Despite these differences, all individual gene analyses clearly support the separation of the genus *Dionda* in two highly differentiated clades with no immediate common ancestor. All mitochondrial and nuclear analyses showed a strongly supported clade formed by seven genera (SW–NM: *Agosia*, *Algansea*, central and southern *Notropis*, *Hybognathus*, *Cyprinella*, *Codoma*, and the southern "*Dionda*" clade), as well as a well-supported but distinct clade consisting of the northern species of *Dionda*. The clade grouping the southern species of genus *Dionda* is more closely related to *Codoma*, as well as to a *Cyprinella* clade, than to the northern species of *Dionda*. The high genetic divergence seen among populations of *Codoma* from different drainages suggests that this putative monotypic genus contains undescribed species. This hypothesis is further supported by the work of Contreras-Balderas (1975), who recognized five races based on morphology, as well as by Mayden (1989), who argued based on field observations of these specimens that they likely represent distinct species. The phylogenetic relationships of *Codoma ornata* has been highly debated; it was considered a member of genus *Cyprinella* based on morphology (Mayden, 1989) and molecular data (Mayden, 2002; although the later study did not include any *Dionda*). However, recent molecular analyses from ribosomal sequences resolved this genus as the sister group to a monophyletic *Dionda* (Simons et al., 2003). The present analysis strongly corroborates a polytypic genus *Codoma* more closely related to the southern "*Dionda*" clade than to a *Cyprinella* clade.

Our analyses showing the polyphyly of *Dionda* are incongruent with the current taxonomy and with earlier allozyme-based studies (Mayden et al., 1992), but are congruent with other sequence-based analyses from mitochondrial gene cyt*b* (Schönhuth et al., 2007; Fig. 2A and B). The generic placement of the species of *Dionda* inhabiting east-central Mexico (Tampico Embayment drainage) has been controversial (Hubbs and Miller, 1977). These later authors presented morphological evidence in support of this "natural grouping" of 6 species as distinct from *Hybognathus*, and treated them only provisionally as members of *Dionda*. Our unexpected recovery of a polyphyletic *Dionda* supports the misgivings that both Hubbs and Miller (R.L.M. pers. comm. with R.R. Miller) had with including these species in *Dionda*. Hence, we propose the natural division of the genus into two different and diagnosable genera. Morphological comparisons of species based on

Table 3
Morphological comparisons of species of "*Dionda*" based on previous descriptions were evaluated to identify morphological features that could be used as diagnostic characters for each clade

Species/ characters	*D. episcopa*	*D. serena*	*D. nigrotaeniata*	*D. diaboli*	*D. argentosa*	*D. melanops*	*D.* sp. (Axtla)	*D. ipni*	*D. erimyzonops*	*D. mandibularis*	*D. dichroma*	*D. catostomops*	*D. rasconis*
Barbels	—	—	—	—	—	—	—	—	—	(−)	+	+	+
Lateral scales	34–45	34–40	36–39	32–36	36–41	34–45	32	32–37	31–34	37–43	37–45	37–40	33–36
Predorsal rows	?	?	?	?	?	?	11	11–14	11–13	17–20	15–23	15–19	14–18
Dorsal origin-pelvic	Above or anterior	Above or anterior	Above or posterior	Slight posterior	Above	Anterior	Above	Over to well behind	Over to well behind	Over to well behind	Over to well behind	Over to well behind	Over to well behind
Anal rays	2, 8	2, 7	I-8	(6–11) 8	?	?	8	9–10 (8–12)	9 (8–10)	(7) 8	(7) 8 (9, 10)	(7) 8	(7) 8 (9)
Ascending process of premaxilla	Slender or absent	Slender or absent	Slender or absent	Slender or absent	Slender or absent	Slender or absent	?	Well developed	Well developed	Well developed	Well developed	Well developed	Well developed
Pharyngeal arch	Stout	Stout	?	?	Stout	Stout	Thick	Heavy	Slender + thick	Heavy	Thick	Slender + thick	Slender
Limps size	Same size	Same size	?	?	Same size	Same size	Lower longer	Lower longer	Lower longer	Lower longer	Lower longer	Equals?? Lower longer	Lower shorter
Upper limp curved	+	+	?	?	+	+	+	+	+	+	+	+	+
Upper limp tip	Pointed	?	?	?	?	?	Pointed	Blunt	Blunt recurved	Pointed recurved	Pointed Recurved	Pointed recurved	Pointed
Teeth (4–4)	Slightly hooked	1th, 4th slightly hooked	?	?	Slightly hooked	Slightly hooked	4 well hooked	4 well hooked	4 well hooked	1st, 2nd well hooked	4th well hooked	4 well hooked	4 well hooked
Lower limb	Curved	Curved	?	?	Curved	Curved	Curved	Straight	Straight	Straight	Straight	Straight	Straight
Grinding surface	+	+	?	?	+	+	+?	+	+	+	+	+	+
Body	Slender	Slender	?	Slender	Slender	Short deep	Frail	Robust	Robust	Slender	Deep	Slender	Slender
Anterodorsal contour arched	+	—	—	—	—	—	+?	+	+	—	—	—	—
Nuptial tubercles	Head	?	?	Head, pectorals	?	?	?	Head, fins	Head, body, fins	Head, body, fins	Head, body, fins	Head, body, fins	Pectoral fin
Drainage	Rio Grande	Nueces-Frio	Colorado and Guadalupe	Rio Grande	Rio Grande	Rio Grande	Pánuco	Pánuco, Tamesí, Coastal	Pánuco, Tamesí	Pánuco	Pánuco	Pánuco	Pánuco
Original description	*Dionda episcopa* Girard, 1856	*Dionda serena* Girard, 1856	*Hybognathus nigrotaeniatus* (Cope, 1880)	*Dionda diaboli* Hubbs & Brown, 1957	*Dionda argentosa* Girard, 1856	*Dionda melanops* Girard, 1856	—	*Notropis ipni* (Alvarez & Navarro, 1953)	*Dionda erimyzonops* Hubbs & Miller, 1974	*Dionda mandibularis* Contreras-Balderas & Verduzco-Martínez, 1977	*Dionda dichroma* Hubbs & Miller, 1977	*Dionda catostomops* Hubbs & Miller, 1977	*Notropis rasconis* Jordan & Snyder, 1899
Synonymized	*Hybognathus flavipinnis*	*Dionda episcopa*	*Dionda episcopa*	—	*Dionda episcopa*	*Dionda episcopa*	—	*Hybognathus rasconis*/ *Dionda rasconis*	*Hybognathus rasconis*	—	*Hybognathus rasconis*/ *Dionda rasconis*	—	*Hybognathus rasconis*/ *Dionda rasconis*

(+), indicates presence of this character; (−), absence; and (?), not described.

previous descriptions were evaluated to identify morphological features that could be used as diagnostic characters for each clade. Girard's original descriptions were superficial by the standards of today, and many of the reportedly diagnostic characters of the various taxa show considerable overlap, (Table 3; Girard, 1859a,b; Cope, 1880; Jordan, 1885; Hubbs and Miller, 1975; Hubbs and Brown, 1957; Hubbs and Miller, 1977; Miller et al., 2005). A more detailed morphological analysis, especially for the Northern clade of *Dionda*, is badly needed. However, the molecular differentiation, phylogenetic relationships and the allopatric distribution observed for northern and southern "*Dionda*" support the recognition of a new genus for the southern species. The type species of *Dionda* is *D. episcopa* (Girard, 1856), and thus the Northern clade retains the name. Based on the combined evidence derived from mtDNA sequences from cyt*b* (herein and Schönhuth et al., 2007) and nuclear genes (herein), the southern lineage of species formerly placed in the genus *Dionda* should be allocated to a new genus. The first described species within this southern clade was *D. rasconis*. This species was first referred to *Notropis* (Jordan and Snyder, 1899), then transferred to *Hybognathus* (Meek, 1904), and later to *Dionda* (De-Buen, 1940). In Hubbs and Miller's (1977) detailed morphological study, *D. rasconis* was included as part of the "natural southern *Dionda* grouping", and their analytical comparisons of the six species of east-central Mexico placed this species close to *D. dichroma*. Unfortunately, *D. rasconis* was not available for the present study, nor in the study by Mayden et al. (1992), but we consider it as part of the southern "*Dionda*" clade. Therefore, based on our molecular analyses (as well as morphological characters; Table 3), we propose a new genus for all species included in the southern *Dionda* clade and endemic to east-central drainages of the Gulf of Mexico.

4.1.1. Tampichthys, new genus

4.1.1.1. Type species. Notropis rasconis Jordan & Snyder, 1899:121, Fig. 3.

Literature cited: Jordan, D.S. and Snyder, J.O., 1899. Notes on a collection of fishes from the rivers of Mexico, with description of 20 new species. Bull. U.S. Fish Comm. v. 19 [1899]: 115–147.

4.1.1.2. Etymology. Tampichthys is derived from the Latinized translation of "fish from the Tampico Embayment drainage of Mexico".

4.1.1.3. Diagnosis. Six species are currently recognized as belonging to *Tampichthys* and were previously referred to the genus *Dionda* (Hubbs and Miller, 1977). These six species can be differentiated from one another by a number of consistent morphological traits outlined by Hubbs and Miller (1977). All these species share the morphological characters of a lateral dark stripe followed by black spot on caudal fin origin, coiled long-gutted intestine, black peritoneum with silvery base, small U-shaped sub-terminal mouth, complete lateral line, tubercles in breeding males, 8 anal rays, well hooked pharyngeal teeth in one row 4–4, head size from 3.5 to 4 times the standard length and dorsal fin origin over to well behind pelvic origin (Fig. 7). Morphological differentiation of species of *Tampichthys* from species of *Dionda* sensu stricto, however, are less consistent. Few morphological characters consistently differentiate *Tampichthys* from *Dionda* (Table 3), despite the fact that these genera are highly differentiated on the basis of molecular evidence and are not close relatives (Figs. 3–5 and Table 2).

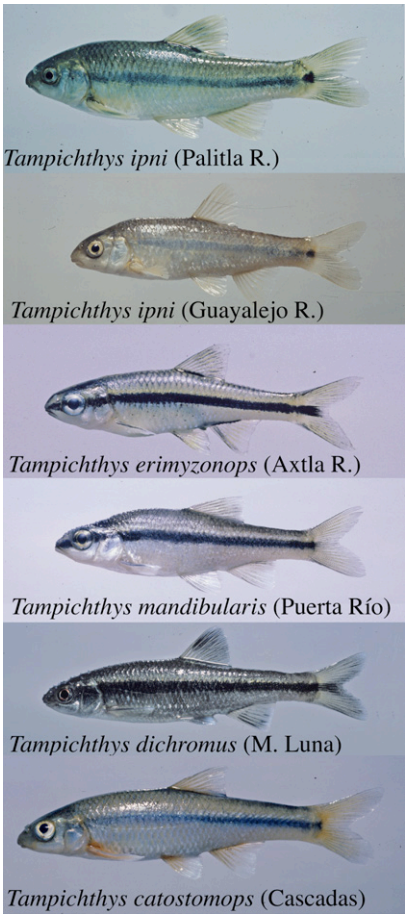

Fig. 7. Life colors of freshly caught specimens of five recognized species of *Tampichthys* photographed by Mayden and Hillis between 1984 and 1987.

This clade exhibits 594 fixed base positions in the 4 genes that can be used to distinguish *Tampichthys* from the other genera: 151 bases fixed in *Tampichthys* that are different in *Dionda*, 31 bases with *Codoma*; 29 with *Cyprinella*; 73 with *Notropis*; 143 with *Campostoma*; 107 with *Algansea*; and 60 with *Hybognathus* (see detailed base positions listed in Appendix B).

4.1.1.4. Included species. Within this newly described genus, the new taxonomic combinations for the six species are:

Tampichthys rasconis (Jordan & Snyder, 1899), *comb. nov.*

T. ipni (Alvarez & Navarro, 1953), *comb. nov.*
T. erimyzonops (Hubbs & Miller, 1974), *comb. nov.*
T. mandibularis (Contreras-Balderas & Verduzco-Martinez, 1977), *comb. nov.*
T. dichromus (Hubbs & Miller, 1977), *comb. nov.*
T. catostomops (Hubbs & Miller, 1977), *comb. nov.*

4.1.1.5. Distribution. These six recognized species of the new genus *Tampichthys* occur in the Tampico Embayment in east-central Mexico at 23°N, and principally inhabit the upper waters on the Pánuco–Tamesí drainage (Fig. 6). Only *T. ipni* is distributed south of the Río Pánuco in five coastal rivers (Tuxpan, Cazones, Tecolutla, Nautla and Misantla rivers) of the eastern stretch of the Trans-Mexican Volcanic Belt (TMB) at 20°N. Genetic divergences among species of this genus (8.5–15.5%) are similar to those found within other recognized genera analyzed here (Table 2).

The results of our molecular analyses are in agreement with previous morphological analyses (Hubbs and Miller, 1977) that considered species of *Tampichthys* to constitute a natural grouping separate from the genus *Dionda*. These results also agree with the previous allozyme analyses of nuclear loci (Mayden et al., 1992) that suggested that the *D. episcopa* complex is a well-supported clade including nine species inhabiting the Rio Grande tributaries and upper Mezquital drainage. The repeatability of the eight clades recovered in our study in mitochondrial and nuclear sequence data sets and the high support for most of these major clades strongly supports this division of *Dionda*.

This new genus comprises three pairs of sympatric species: *Tampichthys ipni* is sympatric throughout much of its range with *T. erimyzonops* in Pánuco and Tamesí drainages; *Tampichthys mandibularis* and *T. dichromus* are sympatric in the upper Río Pánuco although the latter species is more widespread; and *Tampichthys catostomops* and *T. rasconis* are restricted to Río Gallinas (Pánuco drainage). However, the species in each of these pairs occupy different microhabitats. Thus, within this new genus the three pairs of species exhibit a large degree of sympatry, although each species pair is completely allopatric (Hubbs and Miller, 1977). One obvious hypotheses is that sympatric species pairs are the result of sympatric speciation, wherein sympatric members must be sister species. cyt*b* and S7 analyses supported the hypothesis that sympatric species are sister taxa for *T. ipni–T. erimyzonops*, while Rhod and Rag1 do not resolve these intra-generic relationships. There are also some morphological characters that are common between these two species, as they consistency lack barbels, possesses similar body shape, have similar number of scales rows, and share similar shape of the pharyngeal arches (Hubbs and Miller, 1977). *Tampichthys mandibularis* and *T. dichromus* currently exhibit geographical overlap but are not resolved as sister species in any of the analyses. *Tampichthys dichromus* was found to be more closely related to the *T. catostomops*, and *T. mandibularis* was recovered as a highly divergent species in cyt*b*, S7 and Rhod analyses but its relationships were unclear. Previous analytical comparisons of morphology in these six species also concluded that *T. mandibularis* is the most divergent species of the group, while *T. dichromus* shares many characters with *T. catostomops* (Hubbs and Miller, 1977).

Within this genus, *Tampichthys ipni* is the species that exhibits the broadest geographic and ecologic range (from Río Tamesí to Río Misantla). This species also possesses the highest intraspecific mitochondrial genetic divergences within specimens from different drainages (3.7–7.3%), but all separate gene analyses recovered all of these populations in a well-supported clade. These results are in agreement with previous morphological studies that noted some intraspecific local differentiation (Hubbs and Miller, 1977). Interestingly, we found specimens from the Río Guayalejo (Pánuco–Tamesí drainage) more highly divergent than specimens from the Río Nautla (Coastal Plain). Given the combined morphological (Mayden, unpubl. data) and molecular evidence for this species, it is likely that the *T. ipni* clade contains cryptic diversity even within the Pánuco–Tamesí drainage.

All separate gene analyses supported *Codoma* as the closest relative to *Tampichthys*, and together these two genera are closely related to the *Cyprinella* clade, which includes 10 different species inhabiting northern Mexico and southern USA. One incongruence previously cited for the *Cyprinella* clade is that the monophyly of this genus is well-supported in all separate genes except for S7 analyses. In this latter nuclear data set, the two otherwise well-supported clades (C1 and C2) of *Cyprinella* were recovered as paraphyletic. As previously mentioned there are six indels that differentiate these two clades of *Cyprinella*, but five of them are plesiomorphic and are shared with other genera. There are several possible explanations for the topology recovered for *Cyprinella* in S7 analyses. Hybridization is common event between some species within *Cyprinella*, but has never been recorded to occur between any species of *Cyprinella* and species outside of the genus. Alternatively, relationships derived for *Cyprinella* for S7 may be due to difficulty in the alignment of characters due to high variation in indels found in this nuclear region. However, this explanation is not very realistic as the remaining clades discussed above were recovered in congruence with the pattern showed by other genes. Another phenomenon that could explain this pattern could be gene duplication of S7 in some *Cyprinella*, and we have only been able to sequence one of the copies of the gene for one of the sub-clades. If this were correct, it is possible that in this case of *Cyprinella* we compared paralogous sequences for S7, rather than orthologous copies as in other genera. A monophyletic *Cyprinella* is recovered in the other three gene analyses, indicating that the topology recovered in the S7 analyses, in the particular case of this genus, is probably not a reliable species

tree. This highlights the importance of including several genes in a phylogenetic analysis, and supports the point that phylogenetic relationships are more accurate and reliable when there is repeatability of clades derived from different genes.

4.1.2. Genus Dionda

This well-supported clade includes a group of nine northern species of the traditional genus *Dionda*, five of which (*D. argentosa*, *D. melanops*, *D. serena*, *D. nigrotaeniata* and *D.* sp.1 Conchos) had been synonymized with *D. episcopa* until Mayden et al. (1992) elevated these taxa as species. Within this clade, there are six described species (*D. episcopa*, *D. diaboli*, *D. serena*, *D. melanops*, *D. nigrotaeniata* and *D. argentosa*) and three undescribed species (*D.* sp.1, *D.* sp.2 and *D.* sp.3), from the Colorado drainage in Texas to the upper Mezquital drainage in Durango. *Dionda diaboli* is the only described species in this clade not previously synonymized with *D. episcopa*. Three species (*D. serena*, *D. nigrotaeniata* and *D.* sp.1) exhibit allopatric distributions, with *D. serena* confined to the Frio and Nueces river drainages, *D. nigrotaeniata* to the Guadalupe and Colorado drainages, and *D.* sp.1 to the Río Conchos and endorheic Río Nazas drainage. The other six out of nine species in this clade exhibit complex distributions and are in sympatry in parts of their ranges. *Dionda diaboli* has previously been reported from the Devils River system, as well as San Felipe, Sycamore and Pinto creeks (all in Texas). We also discovered *Dionda diaboli* in sympatry with *D. melanops* in upper Río Salado in Coahuila, Mexico. *Dionda melanops* is known to inhabit the Río San Juan in the lower Rio Grande drainage, and this species is presumably the species also found in the Río Salado. *Dionda argentosa* inhabits the Devils River, San Felipe Spring and lower Pecos River, while *D. episcopa* inhabits the Pecos River. Significantly disjunct from any other known populations of the *D. episcopa* complex are two undescribed forms of the upper Mezquital drainage that have a very restricted distributions limited to disjunct areas of Río Tunal (*D.* sp.2 Ojo and *D.* sp.3 Vergel).

Based on previous allozyme analyses, *D. nigrotaeniata* (Colorado and Guadalupe river drainages) and *D. serena* (Nueces River drainage) form the most divergent species for the *D. episcopa* clade. Phylogenetic relationships based on mitochondrial and nuclear genes suggest that *D. diaboli* and *D. serena* are the most divergent species of the present genus *Dionda*, and that the other seven species comprise a well-supported clade. In general, relationships among these seven species are not well resolved with nuclear gene analyses as divergences in these genes were minimal. One consistent problem with current biogeographic and speciation studies is that the current distributions of species may not necessarily be a reliable indicator of the historical ranges of the same species (Losos and Glor, 2003). However, taking into account the phylogenetic relationships among members of the *Dionda episcopa* complex, we postulate that the ancestor of this clade was widely distributed in the historical Rio Grande ranging from Río Tunal (which currently drains to the Pacific Ocean) to the Río San Juan (a tributary of lower Rio Grande). Excluding the genetically more divergent taxa of this clade (*D. diaboli* and *D. serena*), relationships between the remaining seven extant taxa of the *Dionda episcopa* complex suggest that those species that are in closer geographically proximity are closer relatives, an observation that one would expect with allopatric speciation Model I (without intervening extinction of species).

4.2. Faunal inter-drainage dispersal (phylogeography)

The most notable pattern of distribution in North American cyprinids is the faunal distinction between drainages east and west of the Rocky Mountains (Mayden, 1991). In Mexico, the development of one of the word's great tropical–subtropical highlands, bounded by three volcanic mountain ranges (NE: Sierra Madre Oriental; NW: Sierra Madre Occidental; and S: Trans-Mexican Volcanic Belt), has influenced many hydrographic patterns and hence freshwater fish distributions (Miller and Smith, 1986). This complex geological history is the result of orogenic events since the Miocene (Ferrari et al., 2000) and is associated with stream captures, formation of endorheic basins and other drainage modifications that have presumably permitted dispersal and resulted in isolation and speciation in Mexican freshwater fishes (Barbour, 1973a,b; Contreras-Balderas, 1975; Mayden et al., 1992; Doadrio and Dominguez, 2004 Webb et al., 2004; Echelle et al., 2005). All these lines of geological evidence indicate that the historical geographical range of the ancestral species of cyprinids in Mexico and the desert Southwest have changed dramatically over time. Although the continental ice sheets during the Pleistocene glaciation in North America never extended into the study area, these glaciations had some profound indirect effects in freshwater fish faunas in Mexico and are hypothesized to have permitted dispersal by stream captures, local inland or estuarine flooding, and interconnecting drainages due to lowered sea levels during the late Neogene (Conner and Suttkus, 1986). Assuming that the habitat preferences or associations (uplands habitats) of these species and environmental tolerances of these cyprinid species differ little over time, we might expect that their avenues of dispersal were limited (in comparison with more ubiquitous forms) to stream captures or local inland flooding involving headwaters.

Based on the phylogenetic relationships of cyprinid species recovered herein, the current distribution of the species, and the geological history of the region, below we suggest some biogeographic hypothesis for the establishment of current distributions in light of their phylogenetic relationships.

Phylogenetic relationships of the cyprinids examined in this analysis reveal most of the endemic southern and central Mexican clades have sister groups distributed in the Río Grande or more northern rivers. Moreover, some of the major clades recovered herein included both western and eastern groups within clades. This pattern is repeated across clades, with analyses identifying southern with northern taxa: the genus *Algansea* (endemic to central Mexico) is the sister group to *Agosia* (north-western distribution); the newly proposed genus *Tampichthys* (endemic to central-east Mexico) is the sister group of *Codoma* (north-western distribution); and within the *Cyprinella* and *Campostoma* clades. This pattern of distribution, previously characterized for other North American genera by Miller and Smith (1986), was referred to as the Plateau Track and Western Mountain Track. These tracks suggest former hydrographic exchanges across the present arid plateau (as occurs with *Moxostoma*, *Ictalurus* and *Micropterus*) or along the present Sierra Madre Occidental (as occurs with *Gila*, *Campostoma* and *Oncorhynchus*) (Miller and Smith, 1986; Minckley et al., 1986). Confidence in a reconstructed vicariance event (geologic–hydrologic processes) can be garnered from geologic evidence and by observing that multiple monophyletic taxa display similar patterns of sister group relationships (Webb, 1998).

Within *Dionda*, phylogenetic relationships and current distribution patterns of species within this clade suggest a former common ancestor widely distributed across the Rio Grande Region. These phylogenetic analyses support the hypothesis that past tributaries of the Rio Grande reached as far south as the present Río Tunal (upper Mezquital, Mexico), as previously described by other authors (Meek, 1904; Smith and Miller, 1986; Mayden et al., 1992; Echelle et al., 2005). The Río Tunal was later captured from the Rio Grande system by the Río Mezquital and isolated endemic species of *Dionda* and *Codoma* in this Pacific drainage. Moreover, the close relationships among species of *Dionda* from the northern Rio Grande tributaries and South Western Gulf Slope drainages (Frio/Nueces, Colorado and Guadalupe rivers) suggest possible connections between headwaters of these drainages and supports previous hypothesis of headwaters transfers between these drainages based in faunal composition (Smith and Miller, 1986).

Southward on the Mesa Central of Mexico, the main drainage is the Lerma–Santiago river system, separated from Mesa del Norte by the west-trending Sierras Transversales and to the south by Trans-Mexican Volcanic Belt (TMB) (Fig. 6). Based on fish distributions (Miller and Smith, 1986), cladistic biogeography of the Goodeidae (Domínguez-Domínguez et al., 2006), and phylogenetic analyses (Schönhuth et al., 2001; Webb et al., 2004), previous connections between the Río Lerma and northeastern and western drainages have been hypothesized. Interestingly, no species of *Dionda*, *Codoma*, *Tampichthys* or *Cyprinella* are known to inhabit the Lerma drainage. The reduction of volume of lacustrine habitats in the Mesa Central by climatic events or basin capture by headward-eroding streams may have resulted in a late Cenozoic extinction (Miller and Smith, 1986), and could explain the current-day absence of these genera in this area.

Interestingly, close relationships were found between genera, or even between species within different genera, that presently inhabit unconnected drainages and are currently separated by large arid areas. For instance, this pattern occurs with species of *Cyprinella* in arid regions such as the Complejo Guzman (*C. bocagrande* and *C. formosa*) or Nazas and Aguanaval drainages (*C. garmani*) that show close relationships with species of *Cyprinella* from the Pánuco–Tamesí and Rio Grande drainages. A similar pattern is exhibited by the closely related genera *Tampichthys* (endemic to east-central Mexico) and *Codoma* (endemic to north-western Mexico) separated by the Chihuahua desert. Moreover, relationships recovered between species from two distant genera (*Dionda* and *Codoma*) supported the hypothesis that species inhabiting the upper Mezquital (*Dionda* sp. Ojo, *Codoma* sp. Tunal and *D.* sp. Vergel, *C.* sp. Vergel), are closely related with congeneric species that inhabit Conchos, Nazas, Aguanaval, lower Rio Grande tributaries and upper waters of Colorado and Guadalupe drainages. These results support earlier hypotheses arguing that present disjunct populations in arid or semiarid regions reflects partial extirpation from a formerly continuous range due to habitat changes in the recent past and undescribed diversity in *Codoma* (Mayden, unpubl. data).

The relationships we found for central and southern *Notropis* in Mexico are consistent with a series of vicariant events that implicate ancient connections involving the Río Lerma and southern drainages (Balsas and Atoyac). The monophyly of this group suggests a single colonization event of Central and South Mexico and subsequent evolution of different clades. The distribution of this clade is particularly interesting as the various species occur on both sides of the TMB (Fig. 6). Previous paleo-hydrographic hypotheses suggest that the ancient Balsas and Coahuayana drainages reached as far north as current Lerma–Santiago drainage, and volcanic activity in Miocene resulted in the formation of the ancient Lerma and Santiago basins, respectively (De-Cserna and Álvarez, 1995; Gesundheit and Macías-Garcia, 2005). The common ancestor of this *Notropis* clade may have dispersed into these areas, and the subsequent uplift of the Mesa Central could have provided a series of vicariant events responsible for the origins of southern drainages populations and species (Atoyac and Balsas) from Mesa Central drainage populations (Lerma). These vicariant events may have occurred simultaneously with the subdivision of species inhabiting Lerma drainage. This subdivision in the Lerma drainage was also suggested for other group of fishes such as godeids (Domín-

guez-Domínguez et al., 2006) and poecilids (Mateos et al., 2002). Rapid diversification and early in the evolution of species from the Río Lerma (*N. altus*, *N. calientis* and *N. sallaei*) is suggested by only weak support of the internal nodes. However, these hypotheses are in agreement with previous hypotheses of the ancient and successive fragmentation of the Río Lerma drainage across the extensive lacustrine systems of central Mexico (from Miocene to Pleistocene; Miller and Smith, 1986; Gesundheit and Macías-Garcia, 2005) and with *Notropis* fossils from this area (from the Pliocene; Miller and Smith, 1986) that support their ancient presence on the Mesa Central. These results are also in line with the extensive diversification observed within the Goodeidae (Webb, 1998, 2004; Moncayo et al., 2001; Doadrio and Dominguez, 2004) and the Atherinidae (Barbour, 1973a). Furthermore, the close relationships between species from Río Atoyac (*N. imeldae*) with those from Río Balsas (*N. moralesi*, *N. boucardi* and *N.* sp.1) suggest ancient connections between both southern drainages.

It has been suggested that besides Goodeidae, no other strictly freshwater groups, fossil or recent, displays a western United States–Mesa Central disjunction in distribution. Cyprinids and *Chirostoma* have diversified on the Mesa Central as did the Goodeidae, but the first two are related to eastern North American fishes (Schönhuth and Doadrio, 2003; Echelle and Echelle, 1984; respectively). Other groups (*Gila*, *Cyprinodon* and *Fundulus*) extend into Mexico from the United States but none occur on the Mesa Central. The biogeographical implication of our proposed phylogeny (the sister group relationships among cyprinid genera from Eastern Mesa Central or Mesa Central with those of western North America) is that ancestral forms could have been widely distributed in current arid regions. The distribution of several pairs of sister genera (e.g., *Tampichthys*/*Codoma*, and *Algansea*/*Agosia*) support the hypothesis that increasing desiccation during the Tertiary fragmented the range of these ancestral cyprinids, as was previously suggested for other freshwater fishes in the same distribution range as proposed for the families Goodeidae (Webb et al., 2004) and Cyprinodontidae (Echelle et al., 2005).

The SW–NM cyprinid clades occupy habitats in west, central and east of the highlands in Mexico, suggesting an ancient diversification of their common ancestors. This SW–NM clade also supports the hypotheses of connections among different systems, from southern headwaters of the Rio Grande tributaries to Northern headwaters of Atoyac and Papaloapan drainages (*Tampichthys–Codoma*; *Algansea–Agosia*; *Notropis* of the Lerma–Pánuco-*Notropis* of the Atoyac–Balsas–Papaloapan). Moreover, the intra-generic relationships recovered for *Dionda* and *Codoma* are consistent with the recognition of an old Tunal–Nazas–Aguanaval-middle and lower Rio Grande system (Smith and Miller, 1986; Mayden et al., 1992; Echelle et al., 2005). Species of *Algansea* also support historical connections between headwaters of the Ameca and Lerma river drainages, which are also documented by geological data (Smith et al., 1975) and distributions of fish groups (*Notropis*: Chernoff and Miller, 1986; *Chirostoma*: Barbour, 1973b; *Algansea*: Schönhuth, 2002; Goodeidae: Webb, 1998; Doadrio and Dominguez, 2004). The *Notropis* clade also supports connections between the Río Lerma and southern Mexican drainages (Atoyac and Balsas). As we argued previously, the disruptions and fragmentation of these drainages and the subsequent isolation of lineages in new systems can effect and promote speciation within the lineages. On the other hand, the limited sequence divergence recovered among populations of *N. sallaei* from the Pánuco and Lerma headwater drainages in the Mesa Central near Valle de Mexico, and also between populations of *N. moralesi* from southern Mexico drainages (Balsas, Atoyac and Papaloapan), suggest that recent stream capture has been the principal means of passive species dispersal between drainages in central-east Mexico (Chen and Borowsky, 2004). It has been hypothesized that some tributaries to the Río Lerma system drained westward before it was captured by the eastward-draining Río Pánuco drainage (Tamayo and West, 1964; Mateos et al., 2002). The presence of two cyprinid species in the headwaters of the Río Papaloapan (*N. moralesi*) and Río Pánuco (*N. sallaei*) is most likely the result of recent stream piracy events (Schönhuth et al., 2001; Schönhuth and Doadrio, 2003). These dispersal events have left these peripheral populations isolated in headwaters of drainages, providing isolation that may eventually lead to speciation.

Acknowledgments

We thank D. Hendrickson, G. Garrett, C. Norris, C. Hubbs, K. Piller, B. Kuhajda, R. Pérez, G. Pérez-Ponce de León, A. Varela and S. Contreras-Balderas for providing some specimens and especially to D. Neely, H. Espinosa and J.M. Artigaz for their help with the sampling of species. D.W. Nelson for loan specimens of *Notropis* sp. from Río Huichihuayan (UMMZ 238746); A. Bufalino for provide the S7 sequence of *Couesius plumbeus*. We also thank K. Conway, L. Rüber and W.J. Chen for insightful comments on the manuscript. Laboratory work was performed in Mayden's Laboratory of Integrated Genomics, Biodiversity, and Conservation at the Saint Louis University (Missouri, USA) and the Laboratory of Molecular Biology of the Museo Nacional de Ciencias Naturales (Madrid, Spain). This study was funded by the project NSF ATOL Cypriniformes Tree of Life initiative (CToL) to Mayden (NSF Grant EF-0431326) and EX-2005-0106 postdoctoral fellowship from the Spanish Ministerio de Educación y Ciencia to S. Schönhuth. Mayden wishes to acknowledge his sincere gratitude to the late R.R. Miller for his long-term support of his efforts in working with *Dionda* in Mexico and his insights into the group.

Appendix A. Material analyzed

	Locality/drainage	Tissue/voucher	Rag1	Rhod	S7	cyt*b*
Tampichthys						
T. ipni	Río Huichihuayan, North of Tlamaya, Pánuco Dr., San Luis de Potosí, Mexico	CPUM 646	646	646	646	647
	Río Matlapa, at Chalchipepetl, Pánuco Dr., San Luis de Potosí, Mexico	UAIC 7901.05	—	—	—	D8
	Arroyo Palitla, at Palitla, Pánuco Dr., San Luis de Potosí, Mexico	UAIC 9152.01	—	—	—	D848
	Río Bobos, Nautla Dr., Veracruz, Mexico	MNCN 2800, 2809	2800	2800	2800	2800, 2809
	Río Guayalejo at Llera, Tamesí Dr., Tamaulipas, Mexico	UAIC 9148.01	DE843	DE843	843	D11, D843
T. erimyzonops	Río Axtla on Hwy 20, 0.8 km W jct. 120 and 85, Pánuco Dr., San Luis de Potosí, Mexico	UAIC 7900.08	D20	D20	D20	D20
	Río Huichihuayan, Rancho Nuevo, Pánuco Dr., San Luis Potosí, Mexico	MNCN 2931	2931	2931	2931	2931
T. dichromus	La Media Luna System,10 km. S. Río Verde, Pánuco Dr., San Luis de Potosí, Mexico	UAIC 7899.01	D42	D42	D42	D8410
	Spring at Puerta del Río, 20 km SE Cerritos, Pánuco Dr., San Luis de Potosí, Mexico	UAIC 7903.02	—	—	—	D45
	El Aguaje spring, El Aguaje, Pánuco Dr., San Luis de Potosí, Mexico	CPUM 617-618	617	617	617	618
	Charco Azul spring, Near Río Verde, Pánuco Dr., San Luis de Potosí, Mexico	CPUM 726	—	—	726	726
T. catostomops	Las Cascadas, Pánuco, Pánuco Dr., San Luis Potosí, Mexico	UAIC 7898.01				DC4
	Río Tamasopo, Tamasopo, Pánuco Dr., San Luis de Potosí, Mexico	MNCN 2875 CPUM 683	2875	2875	2875	2875 683
	Cascada Tamasopo, Río Gallinas, Pánuco Dr., San Luis de Potosí, Mexico	CPUM 2371	—	—	—	2371
	Río Gallinas, Rascón, Pánuco Dr., San Luis de Potosí, Mexico	MNCN 2865	2865	2865	2865	2865
T. mandibularis	La Media Luna System,10 km. S. Río Verde, Pánuco Dr., San Luis de Potosí, Mexico	UAIC 7899.02	—	—	—	DM13
	Spring at Puerta del Río, 20 km SE Cerritos, Pánuco Dr., San Luis de Potosí, Mexico	UAIC 7903.01	DM29	D53	D29	D53
	Charco Azul spring, Near Río Verde, Pánuco Dr., San Luis de Potosí, Mexico	CPUM 722-724	722	722	724	722
Dionda						
D. diaboli	Pinto Creek, Kinney Co., Rio Grande Dr., Texas, USA	DCP306	—	—	—	DCP306
	San Felipe Creek, Del Rio, Val Verde Co., Rio Grande Dr., Texas, USA	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
	Río San Juan, Melchor Muzquiz, Río Salado, Rio Grande Dr., Coahuila, Mexico	MEX19	MEX19	MEX19	MEX19	MEX19
D. sp. *cf. episcopa 1*	Río San Juan, 26 km S Canutillo on Hwy 45, Conchos Dr., Durango, Mexico	UAIC 7904.01	DE37	DE37	D23	DE37, D23
	Emilio Carranza Dam, Near Emilio Carranza Nazas Dr., Durango, Mexico	CPUM 1949-50	1949	1949	1949	1949–50
D. sp. *cf. episcopa 2 (Ojo de Agua)*	Ojo de Agua de San Juan, Río Tunal, Mezquital Dr., Durango, Mexico	STL 255.01 CPUM 1481	D136 1481	D136 1481	D136 1481	D136 1481
D. sp. *cf. episcopa 3 (Vergel)*	El Vergel spring near Gualterio, Río del Tunal, Mezquital Dr., Zacatecas, Mexico	UAIC 7894.01	D30	D30	D30	D30

(*continued on next page*)

Appendix A (*continued*)

	Locality/drainage	Tissue/voucher	Rag1	Rhod	S7	cyt*b*
D. episcopa	Limpia Creek at Ford Davis, Jeff Davis Co. Pecos River, Rio Grande Dr., Texas, USA	UAIC 12757.01	D32	D32	D32	D32
	Bitter Creek on Bitter Lakes, NWR 6 mi E of Roswell, Rio Grande Dr., New Mexico, USA	STL 110.01	DE110	DE110	—	DE110
	Blue spring 2 mi SW of Black river Village, Eddy Co., Pecos River, Rio Grande Dr., New Mexico, USA	STL 300.1 (8.)	DE300	DE300	DE300	DE300
	El Rico Creek, 1 mi S of Santa Rosa, Guadalupe Co., Pecos River, Rio Grande Dr., New Mexico, USA	STL 659.02	DE659	DE659	DE659	DE659
D. argentosa	Río Escondido, Near Las Cuevas, Coahuila, Rio Grande Dr., Mexico	CPUM 2230	—	—	—	D2230
	Pecos River at Pandale, Val Verde Co., Rio Grande Dr., Texas, USA	UAIC 12755.01	D35	D35	D35	D35
	San Felipe Spring, in Moore Park, Val Verde Co., Rio Grande Dr., Texas, USA	STL 1316	DA0635	DA0635	DA0635	DA0636
D. serena (Nueces/Frio)	Can Creek, at Lost Maples State Park, Bandera Co., Savinal River, Nueces Dr., Texas, USA	UAIC 8348.02 (15.)	DE173	DE173	DE173	DE173
	Paisano Spring, Edwards Co., Nueces Dr., Texas, USA	DSP2806	—	—	—	DSP2806
	Nueces River at Texas Hwy 55 in Barksdale, Real/Edwards Co. Nueces Dr., Texas, USA	STL 1313	DS0633	DS0633	DS0633	DS0633
D. nigrotaeniata (Guadalupe/Colorado)	Headsprigs of Clear Creek, Menard Co., Colorado Dr., Texas, USA	DCC1	DCC1	DCC1	DCC1	DCC1
	Fesenden Spring, Keer Co., Guadalupe Dr., Texas, USA	STL 1311	DSP0631	DSP0631	DSP0631	DSP0631
	Colorado River at municipal Park in Junction, Colorado Dr., Kimble Co., Texas, USA	STL 1314	DSP0634	DSP0634	DSP0634	DSP0634
D. melanops	Cariño de la Montaña stream, Near Ejido Huizachal, Río Salado de los Nadadores, Río Salado, Rio Grande Dr., Coahuila, Mexico	CPUM 566	566	566	566	566
	Río San Juan, Melchor Muzquiz, Río Salado, Rio Grande Dr., Coahuila, Mexico	Mex25	MEX25	MEX25	MEX25	MEX25
	Río San Juan at Allende, 74 km SE Monterrey, Rio Grande Dr., Nuevo León, Mexico	Mex22	MEX22	MEX22	MEX22	MEX22
Related genera						
Agosia						
A. chrysogaster	No locality data.	UAIC 13018.01	—	—	—	SN10
A. sp.1	Río Sonora, Mexico	JEB05-011	AGC05	AGC05	AGC05	AGC05
Algansea						
A. lacustris	Lago Paztcuaro, Lerma Dr., Michoacán, Mexico	MNCN 3025-26	3026	3026	3026	3025-26
		KRP1906	—	—	—	AL19
A. tincella	Presa Orandino, Orandino, Jacona, Lerma Dr., Michoacán, Mexico	MEX09	—	—	—	MEX9
	Laguna Zacapu outlet, Panindicuaro, Lerma Dr., Michoacán, Mexico	MNCN 3450-51	3451	3451	3451	3450
	Presa del Carmen, Santa Marta de los Baños, Lerma Dr., Queretaro, Mexico	MNCN 3307				3307
A. sp. 1	Cañon Coronilla, Ameca Dr., Jalisco, Mexico	MNCN 3696, 3658	3696	3696	3696	3696, 3658
A. avia	Río Compostela, Río Chila Dr., Nayarit, Mexico	SLU 1106.1	AG0906	AG0906	AG0906	AG0906

Appendix A (*continued*)

	Locality/drainage	Tissue/voucher	Rag1	Rhod	S7	cyt*b*
Campostoma						
C. ornatum	Río Sain Alto near Atotonilco, Nazas Dr., Zacatecas, Mexico	UAIC 7895.01	—	—	—	CO879
	Río San Juan, Hwy 45 between El Palmito and Leandro Valle, Durango, Nazas Dr., Mexico	CPUM1640	1640	1640	1640	1640
C. pullum	Río San Juan, Allende, 74 km SE Monterrey, Rio Grande Dr., Nuevo León, Mexico	MEX27	MEX27	MEX27	MEX27	MEX27
C. oligolepis	Emory River at Deermont Rd. in Camp Austin, Morgan Co., Mississippi Dr., Tennessee, USA	STL 888.02	CPO888	CPO888	CPO888	CPO888
C. pauciradii	Snake Creek, 12 miles N County line, Russell Co., Apalachicola Dr., Alabama, USA	UAIC10858.01	CP93107	CP93107	CP93107	CP93107
C. anomalum	Meramec River at MDC access at MO Hwy 8, Crawford Co. Mississippi Dr. Missouri, USA	STL 730.02	CP730	CP730	CP730	CP730
Codoma						
C. ornata	Plan de Ayala stream, Río Tunal, Mezquital Dr., Durango, Mexico	CPUM 1510	1510	1510	1510	1510
	Vergel spring, Río Tunal, Mezquital Dr., Durango, Mexico	CPUM 1455	1455	1455	1455	1455
C. ornata	Río San Juan, at El Cuarto, Aguanaval Dr., Durango, Mexico	CPUM 1528	1528	1528	1528	1528
C. ornata	Peñón Blanco river, at Peñón Blanco, Nazas Dr., Durango, Mexico	CPUM 1659	1659	1659	1659	1659
C. ornata	Isolated pool Arroyo de los Alcoces, Conchos Dr., Chihuahua, Mexico	BRK02-64	CD0264	CD0264	CD0264	CD0264
C. ornata	Río Conchos, Conchos Dr., Chihuahua, Mexico	BRK02-68	CD0268	CD0268	CD0268	CD0268
Cyprinella						
C. alvarezdelvillari	Peñón Blanco, Nazas Dr., Durango, Mexico	MEX33	—	—	—	MEX33
C. bocagrande	Ojo Solo, 2.5 km NE Rancho Nuevo, Guzmán Basin, Chihuahua, Mexico	UAIC 11622.01	CB11622	CB11622	—	CB11622
	Río Bavisque, near Mesa Tres Ríos, Yaqui Dr., Sonora, Mexico	CPUM 893	—	—	893	893
C. formosa	Río Casas Grandes, Guzmán Basin, Chihuahua, Mexico	UAIC 7889.03	CF7889	CF7889	CF01	CF7889
	Río Santa Maria, Guzmán Basin, Chihuahua, Mexico	UAIC 7888.02	—	—	—	CF8702
C. forlonensis	Río Huichihuayan, Rancho Nuevo, Pánuco Dr., San Luis Potosí, Mexico	MNCN 2955	2955	2955	2955	2955
	Río Guayalejo at Llera, Tamesí Dr., Tamaulipas, Mexico	UAIC 7902.02	CLF7902	CLF7902	CF7902	CLF7902
	Río Matlapa, 2 km N of Matlapa, Pánuco Dr., San Luis Potosí, Mexico	UAIC 9153.02	—	—	—	CLF8460
C. garmani	Río Nazas at Hwy 49, Durango, Mexico	UAIC 7891.02	—	—	—	CG7891
	Peñón Blanco, Nazas Dr., Durango, Mexico	CPUM 1468	1468	1468	1468	1468
	Medina stream, Half way between Rio Medina and Jose Maria Morelos, Aguanaval Dr., Durango, Mexico	CPUM 1449	—	—	—	1449
C. lutrensis	Pinto Creek at Hwy 90, Rio Grande Dr., Kinney Co., Texas, USA	UAIC 8352.02	CL8813	CL8813	CL8813	CL8813
C. proserpina	Pecos River at Farm Rd 1024 in Pandale, Rio Grande Dr., Val Verde Co., Texas, USA	STL 1317	C0637	C0637	C0637	C0637
C. sp.1	Río San Pedro at Meoqui, Conchos Dr. Chihuahua, Mexico	UAIC 7909.04	—	—	—	CL7909
	Río San Juan, Río Conchos Dr., Durango, Mexico	UAIC 7904.05	MEX29	MEX29	MEX29	MEX29
	Terlingua Creek, Brewster Co., Rio Grande Dr. Texas, USA	STL1318	—	—	—	CL0638

(*continued on next page*)

Appendix A (*continued*)

	Locality/drainage	Tissue/voucher	Rag1	Rhod	S7	cyt*b*
C. rutila	Río San Juan, Allende, 74 km SE Monterrey, Rio Grande Dr., Nuevo León, Mexico	MEX26	MEX26	MEX26	MEX26	MEX26
C. xanthicara	Río Puente Colorado, Interior Bolsón de Cuatro Ciénegas, Coahuila, Mexico	STL 1097.01	CX92B	CX92	CX92	CX92
Hybognathus						
H. placitus	South Canadian River, Seminole/Pontoc Co., Oklahoma, USA	UAIC 8005.02	HP8799	HP8799	HP8799	HP8799
H. nuchalis	Black River at MO Hwy 49, Wayne Co., Missouri, USA	UAIC 10294.04	SN21	SN21	SN21	SN21
Nocomis leptocephalus	Buffalo River, Wilkinson Co., Mississippi, USA	UAIC 11555.01	SN34	SN34	SN34	SN34
Notropis						
N. imeldae	Río San Francisco, San Pablo Coatlan, Atoyac Dr., Oaxaca, Mexico	MNCN 353-4	354	354	354	353
	Río Agua del Sabino, Sola de Vega, Atoyac Dr., Oaxaca, Mexico	MNCN 1138	—	—	—	1138
N. sp.1	Ojo de Agua de San Miguel Cuevas, Juxtlahuaca, Balsas Dr., Oaxaca, Mexico	MNCN 417-18,	—	420	420	417,418
N. boucardi	Río del Pollo, Colonia Lagunilla, Cuernavaca, Balsas Dr., Morelos, Mexico	MNCN 3487	—	—	—	3487
	Laguna de Huellapan, El Texal, Cuernavaca, Balsas Dr., Morelos, Mexico	MNCN 3474-5	3475	3475	3475	3474
N. moralesi	Río Grande-Verde, Nochixtlan, Atoyac Dr., Oaxaca, Mexico	MNCN 97	—	—	—	97
	Río Grande de San Miguel, Tepelneme de Morelos, Papaloapan Dr., Oaxaca, Mexico	MEX2	MEX2	MEX2	MEX2	MEX2
	Río Igualites, Tlapa, Balsas Dr., Guerrero, Mexico	MNCN 5059	5060	5060	5060	5059
N. sallaei	Río Tula at Ixmiquilpan, Pánuco Dr., Hidalgo, Mexico	UAIC 9151.01	MEX30	MEX30	MEX30	MEX30
	Lerma headwaters, near Jiquipilco, 25 km N Toluca, Lerma Dr., México D.F., México	NC0553	NC0553	NC0553	NC0553	NC0553
	Laguna Almoya del Río, Lerma Dr., México D.F., Mexico	MNCN 3517	—	—	—	3517
N. calientis	Ojo de Agua, San Miguel, Lerma Dr., Michoacán, Mexico	MNCN 3831-3813	3831	3831	3831	3831
	Presa Pirules, San Juan Rayas, Lerma Dr., Queretaro, Mexico	MNCN 3324-26	3324	3325	3324	3326
	Laguna Zacapu, Zacapu, Lerma Dr., Michoacán, Mexico	MNCN 3666, 3717	3717	3717	3717	3666
	Río Mazcua, 8 km W Teocaltiche, Río Verde, Río Grande de Santiago, Lerma Dr., Jalisco, Mexico	SLU1108.01	—	—	—	NC805
Yuriria alta	Presa La Mintzita, La Minzita, Lerma Dr., Michoacán, Mexico	MNCN 3809	3809	3809	3809	
	Laguna Zacapu outlet, Panindicuaro, Lerma Dr., Michoacán, Mexico	MNCN 3449	3449	3449	3449	3449
	Río Juchipila, Jalpa, Río Grande de Santiago, Lerma Dr., Zacatecas, Mexico	MEX15	—	—	—	MEX15
	Río de la Laja, Balneario Xote, Lerma Dr., Guanajuato, Mexico	MNCN 3370	3370	3370	3370	3370
Dionda sp. *cf. ipni*	Río Axtla, on Hwy 120, 0.8 km junct. 120, Pánuco Dr., San Luis de Potosí, Mexico	UAIC 7900.05	D16	D16	D16	D16
Notropis sp.	Río Huichiuayan, Pánuco Dr., San Luis de Potosí, Mexico	UMMZ 238746	NSP238			
Outgroups						
Gila pandora	Rio Chama at US Hwy 84 near Arlequín, Rio Arriba Co., New Mexico, USA	STL 662.01	GP662	GP662	GP662	GP662

Appendix A (*continued*)

	Locality/drainage	Tissue/voucher	Rag1	Rhod	S7	cyt*b*
Couesius plumbeus	Mill Creek, 100 mi N of Fort Nelson, Mackenzie River, British Columbia, Canada	UAIC 11366.01	SN15	SN15	SN15	GB

MNCN, Museo Nacional de Ciencias Naturales, Madrid, Spain; CPUM, Universidad Michoacana de San Nicolás de Hidalgo, Michoacan, Mexico; SLU, Saint Louis University, St. Louis, Missouri, USA; UAIC, University of Alabama Ichthyological Collection, Tuscaloosa, Alabama, USA.

Appendix B

Molecular differences in diagnostic base positions of *Tampichthys* relative to other genera in the four genes analyzed

Gene/genus	cyt*b*	S7	Rhod	Rag1
Dionda	39/C-T; 51/A-C; 52/T-C; 120/T-C; 141/C-AorG; 723/C-T; 883/T-C	8/A-T; 10/T-C; 24/T-A; 33/G-C; 35/C-A; 37/C-T; 39/TA; 47/C-A; 60/C-T; 92/T-GorA; 105/T-A; 143/A-C; 160/G-A; 182/G-A; 215/T-A; 218/G-C; 220/C-T; 254/T-G; 289/A-G; 303/A-T; 317/G-A; 332/G-A; 364/C-T; 379/T-C; 382/A-T; 422/T-C; 427/T-G; 458/A-T; 464/A-G; 474/T-A; 485/T-AorG; 490/G-A; 517/T-G; 529/A-T; 543/A-G; 546/T-G; 558/T-C; 566/G-T; 570/C-T; 573/C-T; 591/T-A; 598/T-C; 605/C-G; 620/T-G; 623/G-A; 645/T-A; 671/A-C; 691/A-T; 692/A-C; 714/A-G; 722/C-A; 724/C-T; 725/A-G; 738/T-A; 749/T-A; 750/C-A; 756/A-T; 771/G-A; 786/A-T; 814/A-T; 817/A-T; 843/G-A; 844/C-T; 848/G-Cor-; 851/A-C; 852/G-A; 853/G-A; 854/G-CorT; 904/C-T; 927/G-A; 928/G-T; 949/T-C; 953/C-G	90/C-T; 93/G-T; 168/C-T; 177/C-G; 196/C-T; 217/T-G; 219/G-C; 234/G-A; 252/C-A; 258/G-T; 267/C-T; 286/C-A; 297/C-Tor-A; 309/C-A; 508/A-C; 510/C; 531/C-T; 534/C-T; 537/C-T; 555/C-G; 558/C-T; 627/C-T; 681/G-C; 688/C-T; 726/G-CorT; 753/C-A; 795/C-G	123/C-T; 309/C-T; 327/G-A; 393/A-G; 412/A-G; 421/G-A; 426/C-GorT; 447/C-T; 453/C-G; 501/C-TorG; 549/GAorT; 552/G-A; 603/C-T; 621/C-T; 663/C-T; 696/A-TorC; 702/GAorC; 714/T-A; 720/T-A; 844/T-A; 882/A-C; 903/T-C; 930/C-T; 1041/G-T; 1053/A-G; 1056/G-A; 1068/G-A; 1092/C-T; 1128/C-T; 1158/G-A; 1170/C-T; 1218/C-G; 1239/A-C; 1266/G-A; 1320/C-T; 1368/C-T; 1404/G-A; 1416/T-C; 1425/C-T; 1434/A-G
Codoma	52/T-C; 117/A-GorC; 381/C-TorA; 513/T-C; 1059/C-T	144/A-C; 219/C-G; 265/A-G; 361/G-T; 406/G-A; 468/A-G; 669/C-T; 727/G-A; 732/C-AorG; 770/T-C; 877/C-G	217/T-A; 219/G-C; 508/A-C; 510/C-T; 825/C-T	720/T-A; 1158/G-T; 1167/A-T; 1215/T-C; 1218/C-G; 1230/C-A; 1240/A-C; 1248/C-T; 1266/G-A; 1309/A-G
Cyprinella	52/T-C; 306/T-C; 594/A-T; 1128/A-CorG	215/T-A; 218/G-C; 566/C-TorGap; 724/C-G; 882/C-T	217/T-A; 219/G-C; 243/T-C; 508/A-C	327/G-A; 393/A-C; 570/A-G; 573/A-C; 840/A-G; 849/T-C; 882/A-C; 1001/A-TorC; 1218/C-G; 1230/C-A; 1247/C-T; 1266/G-A; 1332/A-G; 1404/G-A; 1434/A-G; 1467/T-C
Campostoma	121/T-C; 195/C-A; 210/C; 303/C-AorG; 306/TAorC; 684/C-T; 792/C-T; 819/C-T; 828/T-C; 883/T-C; 1003/C-T	10/T-C; 24/T-A; 35/C-A; 37/C-A; 49/C-A; 51/C-T; 83/G-A; 92/T-G; 106/T-A; 144/A-C; 182/G-A; 194/C-A; 210/G-C; 215/T-A; 218/G-C; 239/T-G; 245/G-A; 260/G-C; 262/A-T; 318/G-A; 364/C-T; 382/A-T; 428/G-A; 432/A-G; 433/A-G; 438/A-G; 446G-A; 456/T-G; 458/A-T; 485/T-A; 495/G-A; 508/C-T; 527/T-G; 557/A-G; 558/T-C; 568C-AorC; 569/G-A; 601/T-A; 630/C-A; 632/T-A; 637/G-A; 670/C-A; 671/A-T; 682/A-CorG; 692/A-G; 693/A-T; 700/G-A; 710/T-A; 714/A-G; 724/C-G; 733/A-G; 738/T-A; 749/T-A; 750/C-A; 761/T-G; 784/A-G; 818/A-T; 842/A-TorG; 845/A-C; 857/A-G; 859/G-AorT; 870/A-C; 871/C-A; 882/C-T; 892/G-C; 928/G-T; 944/T-A; 945/T-C; 951/C-G; 953/C-G	90/C-T; 93/G-A; 168/C-T; 196/C-T; 217/T-A; 219/GCorT; 234/G-A; 252/C-A; 258/G-T; 286/C-A; 462/C-T; 465/T-C; 508/A-C; 510/C-T; 534/C-T; 537/C-A; 555/C-G; 576/G-A; 663/C-A; 726/G-C	42/G-A; 48/C-T; 74/A-T; 87/G-A; 96/G-A; 309/C-T; 339/T-A; 412/A-G; 421/G-A; 447/C-T; 450/T-C; 525/T-C; 549/G-A; 552/G-A; 600/A-C; 603/C-T; 615/C-T; 621/C-T; 633/G-A; 654/T-C; 663/C-T; 666/T-C; 702/G-A; 720/T-G; 804/C-T; 913/T-C; 995/G-A; 1056/G-A; 1068/G-A; 1092/C-T; 1128/C-T; 1158/G-A; 1170C-T; 1218/C-G; 1239/A-C; 1290/T-C; 1368/C-T; 1381/A-G; 1383/A-C; 1404/G-A; 1425/C-T; 1434/A-G

(*continued on next page*)

Appendix B (*continued*)

Gene/genus	cyt*b*	S7	Rhod	Rag1
Notropis	399/C-T	10/T-C; 24/T-A; 36C-A; 38/C-T; 106/T-A; 145/A-G; 176/T-A; 182/G-A; 215/T-CorA; 208/G-C; 245/G-A; 264/T-A; 267/T-G; 360/GCorA; 382A-T; 432/A-T; 458/A-T; 467/G-AorC; 474/T-A; 487/G-T; 506/C-T; 508/C-T; 533/A-G; 601/T-A; 630/C-T; 645/T-A; 671/A-CorT; 680/T-G; 684/C-A; 691/A-C; 692/A-T; 724/C-G; 732/C-G; 738/T-A; 750/C-A; 786/A-TorGap; 815/A-G; 817/A-TorG; 859/G-T; 877/C-T; 882/C-TorGap; 903/G-T; 928/G-T; 945/T-T; 953/C-G	258/G-T; 508/A-C; 510/C-T; 534/C-T; 687/C-T	96/G-A; 309/C-T; 420/G-A; 447/C-T; 549/G-A; 603/C-T; 621/C-T; 702/G-A; 720/T-A; 882/A-C; 1092/C-T; 1004/G-A; 1128/C-T; 1170/C-T; 1239/A-C; 1266/G-A; 1368/C-T; 1404/G-A; 1425/C-T; 1434/A-G; 1467/T-C; 1471/A-C
Algansea	120/C-T; 171/T-GorC; 210/C-T; 252/C-T; 261/A-T; 279/T-C; 399/C-T; 669/C-T; 810/C-AorG; 1041/C-T; 1122/T-C	10/T-C; 24/T-A; 35/C-A; 37/C-T; 93/C-A; 106/T-A; 182/G-T; 215/T-A; 208/G-C; 245/G-A; 251/T-C; 256/A-C; 303/A-G; 364/C-T; 382/A-T; 394/T-C; 410/A-T; 418/C-T; 428/G-A; 455/T-C; 458/A-T; 474/T-A; 506/C-T; 508/C-T; 518/T-C; 540/A-T; 601/T-A; 630/C-TorA; 645/T-A; 671/A-C; 680/T-G; 689/T-G; 691/A-C; 693/A-G; 700/G-A; 724/C-G; 750/C-A; 817/A-T; 859/G-T; 902/T-A; 925/G-C; 928/G-T; 929/T-C; 943/G-T; 953/C-G	93/G-A; 234/G-A; 258/G-T; 286/C-A; 402/C-T; 508/A-C; 510/C-T; 687/C-T; 690/C-A	39/G-A; 48/C-T; 87/G-A; 96/G-A; 213/C-T; 224/T-C; 309/C-T; 327G-A; 412/A-G; 421/G-A; 447/C-T; 549/G-A; 552/G-A; 603/C-T; 612/T-G; 615/C-T; 621/C-T; 702/G-A; 720/T-A; 780/C-A; 804/C-T; 831/T-C; 840/A-T; 846/C-T; 870/C-A; 882/A-C; 903/T-C; 972/T-C; 1068/G-A; 1092/C-T; 1128/C-T; 1170/C-T; 1184/G-A; 1266/G-A; 1368/C-T; 1374/T-C; 1387/A-C; 1401/C-T; 1404/G-A; 1425/C-T; 1434/A-G; 1467/T-C
Hybognathus	669/C-T; 810/C-T; 891/C-T; 1050T-C; 1140/T-C	106/T-A; 115/T-A; 245/G-A; 458/A-T; 508/C-T; 601/T-A; 630/C-A; 879/G-T; 882/C-T; 928/G-T	217/T-A; 219/G-C; 508/A-C; 510/C-T	116/G-C; 116/G-C; 180/T-C; 213/C-T; 219/A-G; 243/G-T; 282/A-T; 309/C-T; 327/G-A; 335/T-C; 339/T-C; 447/C-T; 549/G-T; 570/A-G; 618/T-C; 633/G-A; 637/A-C; 702/G-C; 720/T-A; 726/A-G; 804/C-T; 852/T-C; 882/A-C; 918/T-C; 1001/A-T; 1053/A-G; 1092/C-T; 1119/A-G; 1122/T-C; 1128/C-T; 1143/T-C; 1170/C-T; 1176/A-G; 1213/T-C; 1218/C-G; 1230/C-A; 1301/A-G; 1326/T-C; 1434/A-G; 1443/G-C; 1467/T-C

Numbers indicate base position in the gene and letters indicate the character state observed in *Tampichthys* followed by the character state observed in the other genus. Gaps were treated as missing in the analyses so indels are not included in the appendix.

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