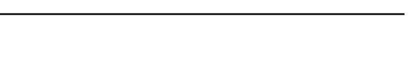

Received: 21 June 2024 | Revised: 23 October 2024 | Accepted: 2 November 2024

DOI: 10.1111/jfb.15999

REGULAR ARTICLE

JOURNAL OF FISH BIOLOGY fsbi

Assessment of genomic diversity within and between two cryptic shiners, the West Texas shiner (*Notropis megalops*) and the Texas shiner (*Notropis amabilis*)

Kayla R. Dye[1] | **Andrew T. Fields**[1] | **Megan G. Bean**[2] | **Sarah M. Robertson**[3] | **Christopher M. Hollenbeck**[1] | **Kevin W. Conway**[4] | **David S. Portnoy**[1]

[1]Department of Life Sciences, Texas A&M University-Corpus Christi, Corpus Christi, Texas, USA

[2]US Fish and Wildlife Service, Austin Ecological Services Field Office, Austin, Texas, USA

[3]Texas Parks and Wildlife Department, San Marcos, Texas, USA

[4]Department of Ecology and Conservation Biology and Biodiversity Research and Teaching Collections, Texas A&M University, College Station, Texas, USA

Correspondence
Kayla R. Dye and David S. Portnoy, Department of Life Sciences, Texas A&M University-Corpus Christi, Corpus Christi, Texas.
Email: kdye1@islander.tamucc.edu and david.portnoy@tamucc.edu

Funding information
Texas Parks and Wildlife Department, State Wildlife Grant Program, Grant/Award Number: F21AFP3731; Texas Sea Grant College Program from the National Sea Grant Office, National Oceanic and Atmospheric Administration, US Department of Commerce, Grant/Award Number: NA22OAR4170092; Texas A&M Agrilife Research, Grant/Award Number: HATCH-TEX09452

Abstract

The presence of cryptic species can hinder effective conservation planning and implementation, as has been the case for speciose groups of freshwater fishes that are difficult to differentiate due to conserved morphologies. The West Texas shiner *Notropis megalops* and the Texas shiner *Notropis amabilis* are a cryptic pair of leuciscids (minnows) that co-occur in spring-fed tributaries of the Rio Grande in Texas and Mexico. Both *N. megalops* and *N. amabilis* are listed as Species of Greatest Conservation Need by the Texas Parks and Wildlife Department. *Notropis amabilis* is widespread and listed as apparently secure by Texas Parks and Wildlife Department whereas *N. megalops* has a very limited distribution and has not been ranked by Texas Parks and Wildlife Department because of data deficiency. Morphological differences between these species have been described; however, proper identification in situ remains problematic. Furthermore, given their range of overlap there is potential for hybridization, and limited genetic data have been collected comparing the species. Therefore, reduced representation genomic and mitochondrial sequencing data were used to reassess the distinctness of the species, screen for hybridization, and characterize their relative frequencies throughout their range of overlap. Genomic analyses recovered two distinct genetic groups corresponding to the species ($F'_{CT} = 0.89$) with no evidence of admixture or introgression. The species were found to co-occur at three sampling locations, two in the Devils River and one in the Pecos, but not in equal frequencies. Overall, these results provide data and tools for further research on *N. megalops* needed for accurate conservation policies and management practices.

KEYWORDS
conservation genetics, diagnostic SNPs, Rio Grande, sympatric, Texas

1 | INTRODUCTION

Extensive flow alteration and stream fragmentation, coupled with other anthropogenic disturbances (e.g., degradation of habitat and water quality, introduction of nonnative species), have contributed to the imperiled status of nearly 40% of North American freshwater fishes (Jelks et al., 2008). However, conservation efforts are often hindered by the complexity of taxonomic relationships and the presence of cryptic species (Pickett et al., 2020). For instance, a species of seemingly low conservation concern with a wide distribution might

© 2024 Fisheries Society of the British Isles.

comprise multiple cryptic species, each with substantially smaller geographic ranges and fewer individuals, and therefore the complex may be of critical yet unrecognized conservation concern (Niemiller et al., 2013). Similarly, a species already of conservation concern could be composed of multiple cryptic species, each rarer than previously thought (e.g., Bickford et al., 2007; Black et al., 2024; Ravaoarimanana et al., 2004). Therefore, proper species delimitation and identification are fundamental to the accurate assessment of biodiversity and biogeography, as well as the implementation of species-specific conservation policies (Quattrini et al., 2019).

The North American genus *Notropis* has long been one of the groups of freshwater fishes for which differentiating among taxa is difficult due to highly conserved morphologies and a large number of species (Stout et al., 2022). The West Texas shiner *Notropis megalops* (Girard, 1856) and the Texas shiner *Notropis amabilis* (Girard, 1856) are an example of a recently recognized cryptic species pair and have partially overlapping distributions in the southwestern United States and Mexico. In 1978, five species originally described as *Alburnus amabilis*, *A. megalops*, *A. socius*, *Cyprinella macrostoma*, and *C. luxiloides* (Girard, 1856) were synonymized into *Notropis amabilis* based on examination of type material (Gilbert, 1978, 1998). However, subsequent analysis of mitochondrial and nuclear genes found unexpectedly high levels of genetic variation within *N. amabilis*, resulting in the reinstatement of *N. megalops* (Conway & Kim, 2016). Despite morphological differences being observed between the two species (Conway & Kim, 2016), proper identification in situ has remained problematic (M. Bean & S. Robertson, pers. comm.).

Notropis megalops and *N. amabilis* are considered Species of Greatest Conservation Need (SGCN) to the Texas Parks and Wildlife Department, with overlapping distributions in the lower Rio Grande drainage (Birdsong et al., 2020; Texas Parks and Wildlife Department, 2023). The lower Rio Grande and its tributaries flow through the arid Chihuahuan Desert region and the waterways have gone through extensive flow alteration and fragmentation for the purpose of human use (Hoagstrom, 2003). Construction of dams and reservoirs for flood control, recreational use, hydroelectric power generation, and irrigation have led to declines in freshwater fishes as these structures block natural migration routes and increase the likelihood of extinction and extirpation by fragmenting populations (Dudley & Platania, 2007). As such, many freshwater fishes within the spring-fed tributaries of the lower Rio Grande exhibit small, highly fragmented distributions and are a priority for conservation (Texas Parks and Wildlife Department, 2012).

Notropis megalops and *N. amabilis* are small, spring-associated fishes with opportunistic lifestyles characterized by short life spans and high mortality at early life stages (Winemiller & Rose, 1992). *Notropis amabilis* is associated with pools and runs over rocky and sandy substrate (Kollaus & Bonner, 2012) and has a protracted reproductive season (9 months) with multiple spawning events taking place within a season (Craig et al., 2017). Despite this, the reproductive mode (e.g., pelagophilic versus lithophilic) remains unknown for *N. amabilis*, and the reproductive biology of *N. megalops* has yet to be studied. However, the closely related Rio Grande shiner *Notropis jemezanus*, sharpnose shiner *Notropis oxyrhynchus*, and emerald shiner *Notropis atherinoides* all belong to a reproductive guild of pelagic broadcast spawning minnows (pelagophils) that produce nonadhesive, semibuoyant eggs (Durham, 2007; Flittner, 1964; Platania & Altenbach, 1998). Fishes belonging to this guild rely on the presence, timing, and duration of suitable stream-flow conditions for successful reproduction (Dudley & Platania, 2007; Durham & Wilde, 2006; Worthington et al., 2018). Opportunistic species have little buffering for stochastic episodic events (e.g., drought) that can lead to within-year recruitment failures, and are therefore highly susceptible to sudden population declines and range contraction (Perkin et al., 2019; Winemiller & Rose, 1992). High levels of extirpation and extinction have been seen in pelagic broadcast spawning minnows throughout the Rio Grande drainage due to high levels of fragmentation of already small geographic ranges (Dudley & Platania, 2007; Osborne et al., 2021).

Notropis megalops is restricted entirely to spring-fed tributaries of the lower Rio Grande in the United States (Texas) and Mexico (Conway & Kim, 2016), and recent efforts have suggested that *N. megalops* is extirpated from multiple smaller tributaries (Sycamore, Las Moras, Pinto, and Live Oak creeks) within the Rio Grande system in Texas (Conway & Kim, 2016). By contrast, *N. amabilis* is widely distributed throughout spring-fed drainages traversing the upper portions of the Edwards Plateau of Texas and throughout the Rio Grande drainage of the United States (Texas and New Mexico) and Mexico (Hubbs et al., 2008; Miller et al., 2005; Page & Burr, 2011), but all records of *N. amabilis* from the lower Pecos River in Texas are thought to be *N. megalops* (Conway & Kim, 2016). In addition, *N. amabilis* was previously reported from the Leona and San Gabriel rivers, Barton Creek, and upper San Antonio River in Texas, and a disjunct population in the upper Pecos River basin in New Mexico, although it is now thought to be extirpated from all of those areas (Conway & Kim, 2016; Craig et al., 2017). Overall, *N. amabilis* is considered uncommon but not rare with some concern over long-term sustainability (apparently secure; NatureServe, 2024) while *N. megalops* has not had its conservation status assessed because of a lack of data.

Given the status and vulnerability of these species, their range overlap, and subsequent potential for hybridization, a robust genomic assessment of *N. megalops* and *N. amabilis* is warranted. Increased resolution provided by genomic approaches can facilitate species delimitation by alleviating shortcomings associated with the use of individual molecular markers (Quattrini et al., 2019), allowing for a high-resolution test of results previously obtained using three markers (one mitochondrial and two nuclear; Conway & Kim, 2016). To accomplish this, double digest restriction-site associated DNA sequencing (ddRAD-seq) was used to genotype individuals at thousands of loci distributed throughout the genome. These data were analyzed to produce a more robust evaluation of species differentiation and explore the range of overlap in the lower Rio Grande drainage. Subsequently, a set of diagnostic single nucleotide polymorphisms (SNPs) were identified that could be used to design an assay for the quick and effective identification of *N. megalops* and *N. amabilis* in future studies. To facilitate a comparison of the results of this study with those of Conway

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and Kim (2016), a subset of individuals was also sequenced using the mitochondrial gene cytochrome *b* (cyt *b*).

2 | MATERIALS AND METHODS

2.1 | Sample collection

A total of 290 individual *Notropis amabilis/megalops* were sampled in 2022 from 11 localities within the tributaries of the lower Rio Grande in Texas. Individuals were captured using seine nets only (25 foot, 3/16th mesh), and tissues (caudal fin clips) were preserved in 20% salt-saturated DMSO buffer (Seutin et al., 1991) and stored at room temperature until the time of DNA extraction using a Mag-Bind® Blood & Tissue DNA Kit (Omega Bio-Tek). A subset of individuals per locality was retained as voucher specimens and fixed in 10% buffered formalin with subsequent transfer to 70% ethanol. Voucher specimens were deposited at the Collection of Fishes at the Biodiversity Research and Teaching Collections, College Station (TCWC; Table S1). Individuals captured within the region of overlapping distributions were not identified to species (i.e., *N. amabilis* or *N. megalops*) prior to sequencing. Additional *N. amabilis* individuals captured beyond the region of overlap (from the Guadalupe River) were obtained from TCWC to include in library preparation and subsequent analysis.

2.2 | Mitochondrial sequencing and phylogenetic reconstruction

The mitochondrial cyt *b* gene was amplified for a subset of individuals using polymerase chain reaction (PCR) and primers LA-*Danio* (GACTYGAARAACCACYGTTG) and HA-*Danio* (CTCCGATCTTCGGATTACAAG; Mayden et al., 2007). Each 25-μL reaction contained 1× buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 μM forward and reverse primers, 1.0 units of GoTaq polymerase (Promega, Madison, WI) and 1.0 μL of DNA template. PCR amplification was run with an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and elongation at 72°C for 1 min. A final round of elongation was run at 72°C for 10 min. Amplicons were purified using Total Pure NGS (Omega Bio-Tek) and bidirectionally Sanger sequenced on an ABI 3730 (Retrogen).

Chromatographs were quality trimmed manually and a consensus sequence was generated for each individual using Geneious v.7.1 (Kearse et al., 2012). Consensus sequences were aligned using clustal omega (Sievers et al., 2020) and edited manually in ugene (Okonechnikov et al., 2012). The resulting alignment contained a total of 1121 base pairs (bp) for 56 individuals. Sequences were also obtained from GenBank for the following taxa: *Agosia chrysogaster*, *Pimephales vigilax*, *Hudsonius hudsonius*, *N. jemezanus*, *N. atherinoides*, *N. percobromus*, and *N. stilbius*. All included GenBank sequences were the same as those included in Conway and Kim (2016) (Table S1), allowing for an assessment of tree similarity across the studies. Novel sequences generated from this study have been deposited in GenBank with accession numbers PQ120597-PQ120652 (Table S1).

Evolutionary distance (*p-distance*) within species and net evolutionary distance (net *p-distance*) between species were calculated using Mega 11 (Tamura et al., 2021) by estimating the mean proportion of nucleotide sites that differ between sequences, while taking into account multiple substitutions at the same site, substitution rate biases, or differences in evolutionary rates among sites between species.

Bayesian analysis was carried out in BEAST 2 v2.7.6 (Bouckaert et al., 2014), with models of nucleotide evolution selected by assessing the Akaike information criterion (AIC), corrected Akaike information criterion (AICc), and the Bayesian information criterion (BIC) outputs from ModelTest-NG and comparing the associated model weights (Darriba et al., 2020; Flouri et al., 2015). Five independent BEAST runs were performed under a strict clock. Each run consisted of 10 million steps with trees sampled every 1000 generations. Tracer v.1.7.2 (Rambaut et al., 2018) was used to confirm adequate run length and effective sample size (ESS). TreeAnnotator v.2.5 was used to obtain the maximum clade credibility tree and estimate posterior probabilities for each clade.

2.3 | ddRAD-seq library preparation and analysis

Preparation of ddRAD libraries followed a modified version of Peterson et al. (2012). High molecular weight DNA obtained from extraction was digested using enzymes *Msp*I and *Eco*RI. The digested fragments were standardized to 100 ng and barcoded adapters were ligated to the *Eco*RI sites, while a common adapter was ligated to the *Msp*I sites. After adapter ligation, individuals were combined into index-specific pools and size selected (313–437 base pairs) using a Pippin Prep size-selection system (Sage Science). Flow cell adapters with one of four index identifiers were incorporated to each set of pooled individuals ($n = 42$–46) using 14 cycles of PCR and indexed pools were combined into libraries of approximately 175 individuals which were sequenced across two lanes of an Illumina HiSeq 4000 (paired end 150 bp). Libraries contained a mix of individuals across species and locations, and included duplicate individuals (technical replicates).

Following sequencing, individuals were demultiplexed using barcode and index sequences with *process_radtags* (Catchen et al., 2011), and the dDocent pipeline was used for de novo reference construction, read mapping, and SNP calling (Puritz et al., 2014). A multi-species de novo reference was assembled using 20 individuals identified to species using the results of aforementioned mitochondrial analysis (10 *N. amabilis* and 10 *N. megalops*). Raw SNP variants and individuals were filtered for quality using vcftools v0.1.14 (Danecek et al., 2011) and R functions in a customized workflow, following practices laid out in O'Leary et al. (2018). Filtering initially removed indels, loci with <2 alleles, quality score <20, and depth <5 reads. Loci were further filtered based on allele balance, depth/quality ratios, strand representation, paired read representation, missing data,

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

high variation in depth across a locus within an individual, minimum allele frequency, and excess locus heterozygosity to remove potential paralogs and other technical artefacts. In the final dataset, retained loci had a mean depth >20, were called in at least 90% of individuals, and individuals had less than 20% missing data. Microhaplotypes were then generated by collapsing SNPs on the same contig to produce a dataset of multi-allelic SNP-containing loci (Willis et al., 2017). Contigs with an excessive number of SNPs (>75% quantile) were removed. In addition, the composite genotypes of technical replicates included within and across libraries were compared to characterize locus-specific genotyping error. One individual from each pair of replicates was removed. To minimize genotype inconsistencies across libraries (i.e., library effects), individuals were grouped by index and library and two methods were used to identify and remove loci contributing to differences among libraries. The first method assumes an island model and utilizes a Bayesian approach to generate a null distribution of F_{ST} for neutral loci in BAYESCAN (Fischer et al., 2011; Foll et al., 2010; Foll & Gaggiotti, 2008). This method was run with prior odds of 1000 and a burn-in of 200,000 iterations; 20 pilot runs of 5000 iterations were used to tune MCMC parameters and following 30,000 sampling iterations with a thinning interval of 50, significance was evaluated using a *q* value of 0.05. The second method utilizes an inferred distribution of neutral F_{ST}, after trimming the highest and lower 5% of F_{ST} values to identify F_{ST} outliers in *OutFLANK* (Whitlock & Lotterhos, 2015). Significance was evaluated using a *q* value of 0.05. Full details of filtering and data quality control are available at https://github.com/marinegenomicslab/notropis_gen_diversity.

Genetic variation was assessed using an unsupervised clustering algorithm (*K*-means) that partitions the data into a given set of *k* clusters and classifies the individuals by minimizing within cluster diversity and maximizing between cluster diversity. Identified genetic clusters were tested using discriminant analysis of principal components (DAPC) with cross-validation in the R package *adegenet* (Jombart, 2008). This method optimizes the number of PCs in the model by dividing the data into a training set and validation set, with the validation set being selected via stratified random sampling. DAPC is carried out on the training set with variable numbers of PCs retained, and the degree to which the analysis is able to accurately predict the group membership of excluded individuals (those in the validation set) is used to identify the optimal number of PCs to retain (Jombart et al., 2010). A *K*-means clustering analysis was performed for $K = 2$ (i.e., species) and validated using 50 repetitions at each level of PCs retained for a maximum of 300 PCs.

The data was further screened for potential evidence of current hybridization using two methods. The first method, NEWHYBRIDS, is a Bayesian clustering method that estimates the posterior probability that an individual belongs to a pure species or hybrid genotype class (Anderson & Thompson, 2002). Posterior probabilities were calculated for five genotype classes: pure *N. amabilis*, pure *N. megalops*, F_1 hybrid, *N. amabilis* backcross, and *N. megalops* backcross. Five independent runs were conducted using the default parameters on 150 randomly selected SNPs. The second method utilized the hybridize function of *adegenet* to derive allele frequencies for each species and sample the subsequent gametes using a multinomial distribution (Jombart, 2008). Mitochondrially identified individuals were used to simulate the F_1 hybrid, *N. amabilis* backcross, and *N. megalops* backcross.

To determine degree of genetic differentiation, a hierarchical AMOVA was run in ARLEQUIN v3.5 (Excoffier & Lischer, 2010), with individuals first grouped by species, based on results of *K*-means clustering, and then by sampling location. Significance was determined using 10,000 permutations at an α of 0.05. To overcome the restriction on the magnitude of fixation indices caused by background diversity (Hedrick, 2005), the observed fixation index between the species (F_{CT}) was standardized by the maximum value that could be obtained, given observed levels of genetic variation, using the method laid out by Meirmans and Hedrick (2011). Briefly, alleles for *N. megalops* were re-coded in a way that no alleles were shared between species. For example, at a given locus, alleles shared between the species would be named 40424_001 and 40424_003, after recoding, the alleles for *N. megalops* would be named 40424_101 and 40424_103, creating unique alleles for that species. Using the re-coded dataset, another hierarchical AMOVA was run as above to determine the maximum value of $F_{CT(max)}$. The standardized fixation index was then calculated as:

$$F'_{CT} = \frac{F_{CT}}{F_{CT(max)}}.$$

Finally, the frequency of each species was characterized at each of the 11 discrete sampling locations spread across the lower Pecos River and its tributary Independence Creek, the Devils River, San Felipe Creek, and Pinto Creek.

2.4 | Identification of diagnostic SNPs

A subset of diagnostic SNPs was identified that could be used to distinguish *N. megalops* and *N. amabilis* either using a probe-based qPCR assay (Heid et al., 1996) or a genotyping in thousands by sequencing (GT-seq) panel (Campbell et al., 2015). The SNPs were selected by identifying those that were completely fixed between the species on loci shared between the two species. The subset was then filtered to remove SNPs within 30 bp of the beginning and end of the contig to allow room for future primer design, and to retain contigs with SNPs within 20 bp of each other to allow for multiple bases to be contained within a qPCR probe, potentially enhancing probe specificity.

2.5 | Ethics Statement

All voucher specimens and tissue samples (caudal fin clips) were acquired following appropriate animal care standards of the individual Federal and State agencies involved, or approved animal care protocols at affiliated academic institutions (Portnoy, TAMUCC IACUC 2023–0022; Conway, TAMU IACUC 2023–0216).

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

3 | RESULTS

3.1 | Phylogenetic reconstruction

Phylogenetic analysis contained a subset of 56 individuals from this study (*Notropis megalops*, $n = 31$; *N. amabilis*, $n = 25$) and nine GenBank sequences including *N. percobromus* ($n = 1$), *N. atherinoides* ($n = 2$), *N. jemezanus* ($n = 2$), *N. stilbius* ($n = 1$), *P. vigilax* ($n = 1$), *H. hudsonius* ($n = 1$), and *A. chrysogaster* ($n = 1$). The final cyt *b* alignment consisted of 1121 bp with no gaps. Estimation of models of nucleotide evolution from ModelTest-NG resulted in TrN + I + G being the best model according to BIC with a score of 0.5655 (Darriba et al., 2020). AIC and AICc resulted in alternate models with lower scores, 0.4171 and 0.3139, respectively. Model TrN + I + G was chosen as the final model based on the compared scores and was used for subsequent analysis.

Bayesian analysis resulted in a monophyletic group comprising all members of *Notropis* included in this study with 100% posterior probability support. Within the *Notropis* clade, *N. megalops* was sister to all remaining members, and *N. amabilis* was sister to a clade including *N. atherinoides*, *N. jemezanus*, and *N. stilbius* (Figure 1). For the segment of cyt *b* investigated, the net *p*-distance between *N. amabilis* and *N. megalops* was 13.4% (Table 1). Comparatively, the putative sister taxa *N. amabilis* and *N. jemezanus* had a net *p*-distance of 7.1%. Intraspecific genetic differences for *N. amabilis* and *N. megalops* were 1.68% and 0.25%, respectively (Table 1).

3.2 | ddRAD-seq data analysis

After demultiplexing and trimming, the mean number of reads per individual was 2,162,335 across both libraries. Filtering removed four

TABLE 1 Mean genetic distances within (diagonal) and between (below diagonal) *Notropis amabilis* ($n = 26$), *N. megalops* ($n = 34$), and *N. jemezanus* ($n = 2$), estimated using the 1121 bps of the mitochondrial cytochrome *b* gene.

	N. amabilis	***N. megalops***	***N. jemezanus***
N. amabilis	0.01678		
N. megalops	0.1347	0.002529	
N. jemezanus	0.07129	0.1506	0.006244

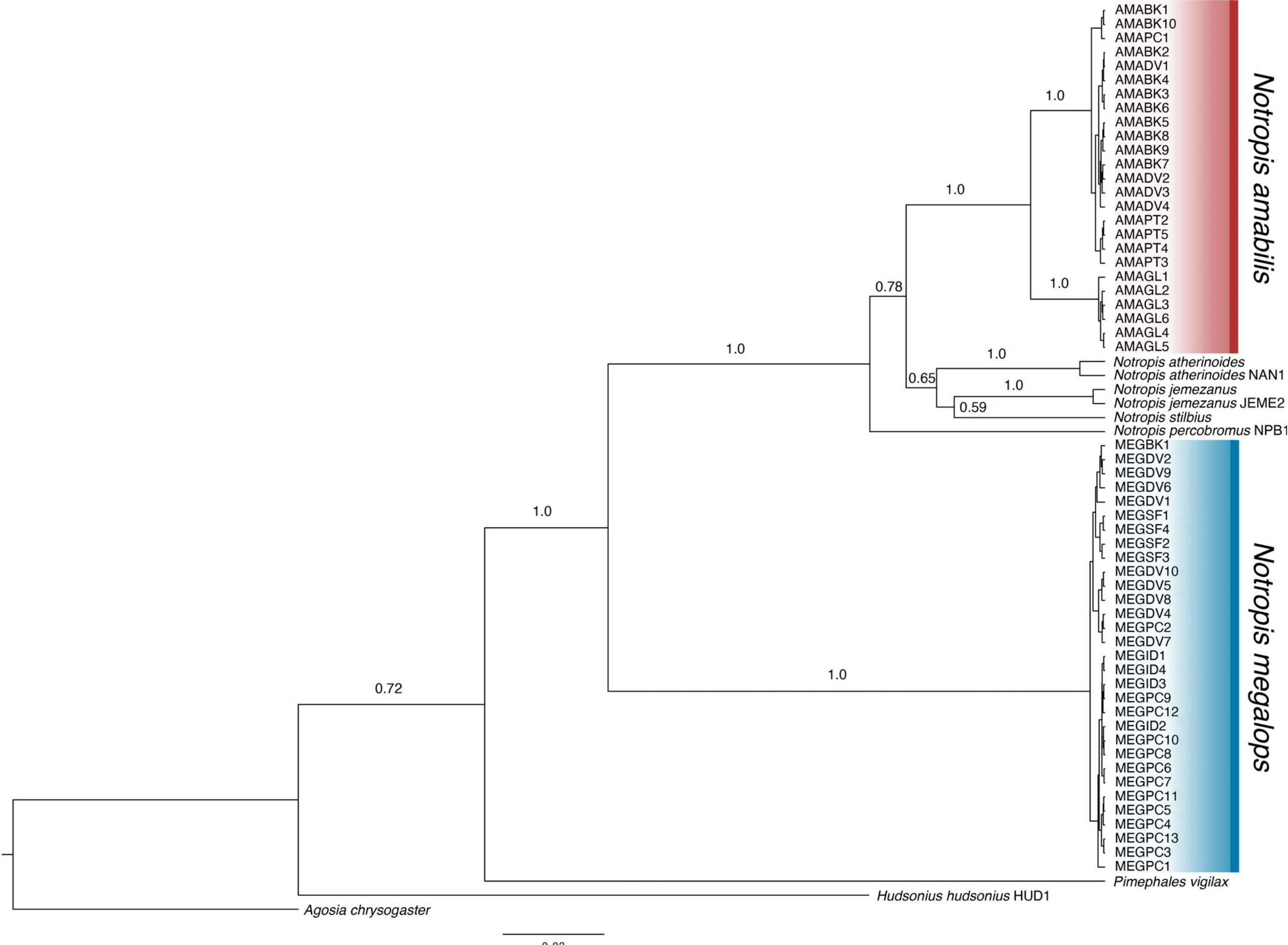

FIGURE 1 Phylogenetic tree derived from Bayesian analysis of the 1121 bp fragment of mitochondrial gene cytochrome *b* (*Notropis megalops*, $n = 31$; *N. amabilis*, $n = 25$). Numbers above branches represent posterior probabilities.

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

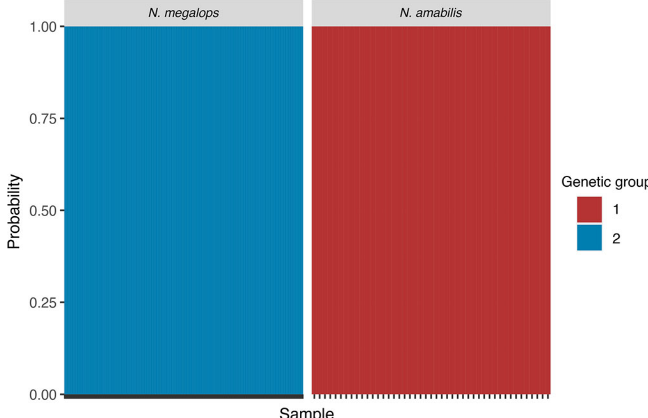

FIGURE 2 Discriminant analysis of principal components using the unsupervised clustering algorithm, *K*-means (= 2), using 3742 single nucleotide polymorphism-containing loci and 286 individuals (*Notropis megalops*, $n = 241$; *N. amabilis*, $n = 45$).

individuals with >20% missing data. Assessment for library effects removed 10 unique putative outlier loci (six identified by *OutFLANK* and eight identified by BayeScan). After filtering, the final data set contained 286 individuals genotyped at 20,771 SNPs spread across 3742 SNP-containing loci, with a mean of 5.55 SNPs and 3.66 alleles per locus.

For $K = 2$, cross-validation for DAPC returned 100% assignment for the two genetic groups (i.e., species) regardless of the number of PCs tested (Figure 2). The genetic groupings corresponded to the mtDNA species identification for the subset of individuals that were included in both analyses, with no evidence of mtDNA introgression. Neither method used to detect hybridization detected any past or present interaction between the species. The five independent runs conducted by NewHybrids resulted in 100% assignment of each individual to one of the two species (Table S2). Similarly, the allele frequency-based simulation using *hybridize* assigned each individual to one of the two species (Figure S1).

Components of variance were significant at all levels for the hierarchical AMOVA, with 4.98% of the variance explained by population structure within species ($F_{SC} = 0.187$, $p < 0.001$) and 73% ($F_{CT} = 0.734$, $p < 0.001$) explained by difference between the species (Table S3). The maximum value for F_{CT} was 0.821 resulting in an F'_{CT} of 0.894.

In the Pecos River, across six sampling locations (inclusive of its tributary Independence Creek) one individual out of 156 collected was *N. amabilis* and the remainder were *N. megalops*. In the Devils River, across three sampling locations, 24 individuals out of 85 were *N. amabilis* and the remaining 61 were *N. megalops*. Within the Devils River there were two sampling locations where both species were found; at Bakers Crossing (Val Verde County, TX) one sample out of 20 collected was *N. megalops* and at the Devils River and Dolan Creek confluence (Val Verde County, TX) five samples out of 26 were *N. amabilis* (Figure 3).

3.3 | Identification of diagnostic SNPs

Filtering for diagnostic SNPs identified 6223 SNPs completely fixed different on loci shared between the species. Further filtering for future panel utilization resulted in 521 diagnostic SNPs at 210 microhaplotype loci. The power of the selected diagnostic markers to correctly assign each individual to a distinct species was investigated using *K*-means clustering for $k = 2$. Cross-validation for DAPC indicated that there was 100% assignment to two genetic groups.

4 | DISCUSSION

The present study highlights the importance of a genomic approach for proper species differentiation and identification in support of species-specific conservation policies for *N. amabilis* and *N. megalops*. Mitochondrial and genomic data generated in this study support the findings of Conway and Kim (2016), that *N. amabilis* and *N. megalops* are two distinct species. The results of the cytochrome *b* analysis in this study recover *N. megalops* as sister to all remaining *Notropis* species included in the dataset, with *N. percobromus*, *N. atherinoides*, *N. jemezanus*, and *N. stilbius* nestled between *N. megalops* and *N. amabilis* within the *Notropis* clade (Figure 1). The independence of the two species is further supported by a net *p*-distance greater between *N. megalops* and *N. amabilis* than between *N. amabilis* and *N. jemezanus* (Table 1). The results of population genomic analyses also demonstrate two distinct genetic groups, with no evidence of interaction or hybridization, supported by cross-validation and an F'_{CT} value (0.89) close to complete fixation.

Recent research suggests that both *N. megalops* and *N. amabilis* are extirpated from multiple historic reaches (Conway & Kim, 2016; Craig et al., 2017), and following the reinstatement of *N. megalops*, *N. amabilis* was no longer considered to occur within the lower Pecos River (Conway & Kim, 2016). However, the present study recovered a single *N. amabilis* in the upper-most sampling location on the Pecos River (Val Verde County, Texas; Figure 3). This indicates that *N. amabilis* and *N. megalops* still co-occur in the lower Pecos River. However, with only one *N. amabilis* present in more than 150 individuals sampled, understanding the species frequency and distribution in the lower Pecos will require further sampling. Field sampling also revealed two other sites of sympatry within the Devils River, at Bakers Crossing (Val Verde County, Texas; Figure 3) and the confluence of Devils River and Dolan Creek (Val Verde County, Texas; Figure 3). Only one *N. megalops* was caught at Bakers crossing, however, at the confluence of the Devils River and Dolan Creek multiple individuals of both species were encountered (*N. megalops*, $n = 21$; *N. amabilis*, $n = 5$; Figure 3). Pinto Creek was historically included in the region of overlap between these species (Conway & Kim, 2016; Garrett et al., 2004), but field sampling for the present study only recovered *N. amabilis* at one site in the upper reaches of Pinto Creek. Overall, the frequency of each species throughout their region of overlap suggests that they co-occur more often than previously thought. Although this is a robust assessment of the region of overlap

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

FIGURE 3 Lateral view of (a) *Notropis megalops*, in life, Texas Cooperative Wildlife Collecion (TCWC) 16455.05, male, ~ 40.0 mm standard length (SL); USA: Texas: San Felipe Creek and (b) *Notropis amabilis*, in life, TCWC 16879.08, 38.0 mm SL; USA: Texas: Frio River. (c) Sampling locations of *Notropis* individuals throughout the range of overlap included in this study. Independence Creek (*N. megalops*, *n* = 32), Pecos River (*N. megalops*, *n* = 121; *N. amabilis*, *n* = 1), Devils River (*N. megalops*, *n* = 59; *N. amabilis*, *n* = 24), San Felipe Creek (*N. megalops*, *n* = 29), Pinto Creek (*N. amabilis*, *n* = 14), Guadalupe River (*N. amabilis*, *n* = 6).

of these species, sampling was not able to take place in Sycamore Creek, a Rio Grande tributary that both species were previously observed (Conway & Kim, 2016), due to the creek running dry. The potential region of overlap within the Mexico portions of their distributions (Conway & Kim, 2016) were also not explored, so it is possible that the range of *N. megalops* is larger than is included in this study, and that the two species may occur sympatrically in more than just the tributaries and stretches sampled here.

As spring-associated species, *N. megalops* and *N. amabilis* rely on clear, flowing water for successful life cycles. Within the overlapping range of these species, the Amistad reservoir lies across the Rio Grande, inundating stretches of the Pecos and Devils Rivers upstream, decreasing flow downstream, and restricting movement between the upper and lower portion of this range (Calamusso et al., 2005; Figure 3). The extensive fragmentation of the Rio Grande has led to a dramatic decline for numerous native pelagophils resulting in federal or state protection (e.g., Pecos bluntnose shiner *N. simus pecosensis* federally threatened; Rio Grande silvery minnow *Hybognathus amarus* federally endangered; Rio Grande shiner *N. jemezanus* state threatened; Dudley & Platania, 2007; Osborne et al., 2021; Texas Parks and Wildlife Department, 2023) or extinction (e.g., the phantom shiner *N. orca*; the Rio Grande bluntnose shiner *N. simus simus*; Bestgen & Platania, 1990). Nearly half of the fishes native to the Chihuahuan Desert region are ranked as Endangered, Vulnerable, or Near-threatened (Perkin et al., 2022). The distribution of *N. megalops* closely matches that of two other Rio Grande endemics, the Proserpine shiner *C. proserpina*, and the Rio Grande darter *Etheostoma grahami*, both of which are listed by Texas Parks and Wildlife Department as threatened, as a result of habitat loss and fragmentation, decreased stream flows, and alteration of flow regime in the lower Rio Grande drainage (Jelks et al., 2008). While the majority of the range of *N. amabilis* lies throughout the drainages traversing the Edwards Plateau of Texas (Hubbs et al., 2008; Miller et al., 2005; Page & Burr, 2011), *N. megalops* is almost entirely restricted to the tributaries of the lower Rio Grande, with a possible fragmented distribution in the Trans-Pecos region of Texas and Nuevo Leon, Mexico (Conway & Kim, 2016). Given the demand for freshwater, for irrigation and human consumption, throughout arid regions of the Chihuahuan Desert, water availability and quality may continue to decline and further increase the vulnerability of opportunistic species endemic to these waters.

Given the fragmented distribution across an already small geographic range and the status of sympatric species or those with presumably similar ecologies and life histories, a species-specific

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

conservation assessment is warranted for *N. megalops*. However, the conserved morphologies between *N. amabilis* and *N. megalops* make in situ identification exceedingly difficult. The 210 diagnostic loci identified allow for creation of a reliable, nonlethal genomic tool that can be used to differentiate between these similar species using economically efficient methods like qPCR (Heid et al., 1996) and GTseq (Campbell et al., 2015). These tools could be used to efficiently identify large numbers of adults and juveniles for a more complete view of distribution and relative frequency, or larvae necessary for assessments of reproductive ecology, promoting species-specific conservation planning.

AUTHOR CONTRIBUTIONS

Ideas: M.G.B., S.M.R., K.W.C., and D.S.P. Data generation: K.R.D., M.G.B., S.M.R., K.W.C., and D.S.P. Data analysis: K.R.D., A.T.F., C.M.H., and D.S.P. Manuscript preparation – original draft: K.R.D. Manuscript preparation – review and editing: A.T.F., M.G.B., S.M.R., C.M.H., K.W.C., and D.S.P. Funding: K.R.D., M.G.B., S.M.R., K.W.C., and D.S.P.

ACKNOWLEDGEMENTS

The authors would like to thank Texas Parks and Wildlife Department, Amanda Pinion, Elizabeth Hunt, Kole Kubicek, Katrina Keith, and the Riverscape Ecology Lab at TAMU-College Station for assisting with tissue collection. We also would like to thank the Nature Conservancy, the US Fish and Wildlife Service, and all individual private land owners as without access to their land this project would not have been possible. We also wish to thank the Marine Genomics Lab for helpful discussion. The findings and conclusions in this article are those of the author(s) and do not represent the official views of the US Fish and Wildlife Service. This is publication 40 of the Marine Genomics Laboratory and 132 of Genetic Studies in Fishes, and publication 1696 of the Biodiversity Research and Teaching Collections at Texas A&M University.

FUNDING INFORMATION

Research was funded by Texas Parks and Wildlife Department, State Wildlife Grant Program (F21AFP3731) and supported in part by an Institutional Grant NA22OAR4170092 to the Texas Sea Grant College Program from the National Sea Grant Office, National Oceanic and Atmospheric Administration, US Department of Commerce A&M Agrilife Research (Hatch TEX09452).

DATA AVAILABILITY STATEMENT

R scripts are available at https://github.com/marinegenomicslab/notropis_gen_diversity. Mitochondrial DNA sequences are available on GenBank with accession numbers PQ120597-PQ120652 found in Table S1. Genomic data is available through the NCBI SRA BioProject number PRJNA1147701.

ORCID

Kayla R. Dye https://orcid.org/0000-0002-8701-9446
Andrew T. Fields https://orcid.org/0000-0002-2817-2141
Megan G. Bean https://orcid.org/0000-0001-7017-1153
Christopher M. Hollenbeck https://orcid.org/0000-0003-0227-7225
Kevin W. Conway https://orcid.org/0000-0002-0161-8385
David S. Portnoy https://orcid.org/0000-0002-8178-1018

REFERENCES

Anderson, E. C., & Thompson, E. A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, *160*, 1217–1229.

Bestgen, K. R., & Platania, S. P. (1990). *Extirpation of Notropis simus simus (Cope) and Notropis orca Woolman (Pisces: Cyprinidae) from the Rio Grande in New Mexico, with notes on their life history* (Vol. 6, pp. 1–8). Occasional Papers of the Museum of Southwestern Biology, University of New Mexico.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*, 148–155.

Birdsong, T., Garrett, G., Bean, M., Bean, P., Curtis, S., Fleming, P., Grubh, A., Lutz-Carrillo, D., Mayes, K., Robertson, C., Robertson, S., Schlechte, J. W., & Smith, N. (2020). Conservation of Texas freshwater fish diversity: selection of Species of Greatest Conservation Need. Texas Parks and Wildlife Department. PWD RP T3200-2780 (10/20). Austin, Texas.

Black, A. N., Heenkenda, E. J., Mathur, S., Willoughby, J. R., Pierce, B. L., Turner, S. J., & Rizzuto, D. (2024). Rapid vertebrate speciation via isolation, bottlenecks, and drift. *Evolution*, *121*, e2320040121.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *10*, e1003537.

Calamusso, B., Rinne, J. N., & Edwards, R. J. (2005). Historic changes in the Rio Grande fish fauna: Status, threats, and management of native species. *American Fisheries Society Symposium*, *45*, 205–223.

Campbell, N. R., Harmon, S. A., & Narum, S. R. (2015). Genotyping-in-thousands by sequencing (GTSeq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources*, *15*(4), 855–867.

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: Building and genotyping loci de novo from short-read sequences. *G3 Genes|Genomes|Genetics*, *1*, 171–182.

Conway, K., & Kim, D. (2016). Redescription of the Texas shiner *Notropis amabilis* from the southwestern United States and northern Mexico with the reinstatement of *N. megalops* (Teleostei: *Cyprinidae*). *Ichthyological Exploration of Freshwaters*, *26*, 305–340.

Craig, C. A., Littrell, B. M., & Bonner, T. H. (2017). Population status and life history attributes of the Texas shiner *Notropis amabilis*. *The American Midland Naturalist*, *177*, 277–288.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., Durbin, R., & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*, 2156–2158.

Darriba, D., Posada, D., Kozlov, A. M., Stamatakis, A., Morel, B., & Flouri, T. (2020). ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution*, *37*, 291–294.

Dudley, R. K., & Platania, S. P. (2007). Flow regulation and fragmentation imperil pelagic-spawning riverine fishes. *Ecological Applications*, *17*, 2074–2086.

Durham, B. W. (2007). Reproductive ecology, habitat associations, and population dynamics of two imperiled cyprinids in a Great Plains River (Doctoral thesis, Texas Tech University, TX). Retrieved from: https://ttu-ir.tdl.org/items/2137d33d-151e-436e-8482-4aa52d9ec3fb

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Durham, B. W., & Wilde, G. R. (2006). Influence of stream discharge on reproductive success of a prairie stream fish assemblage. *Transactions of the American Fisheries Society*, *135*, 1644–1653.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and windows. *Molecular Ecology Resources*, *10*, 564–567.

Fischer, M. C., Foll, M., Excoffier, L., & Heckel, G. (2011). Enhanced AFLP genome scans detect local adaptation in high-altitude populations of a small rodent (*Microtus arvalis*). *Molecular Ecology*, *20*, 1450–1462.

Flittner, G. (1964). *Morphometry and life history of the Emerald shiner, Notropis atherinoides, Rafinesque* (Doctoral thesis, University of Michigan, MI). Retrieved from: https://www.proquest.com/docview/302138764/fulltextPDF/2805A4264A7D4473PQ/1?%20Theses&accountid=7084&parentSessionId=iOmYK5eLjxosjDrAqORCWfuqMVxu5ruiGwZ4bfHYBxU%3D&sourcetype=Dissertations%20

Flouri, T., Izquierdo-Carrasco, F., Darriba, D., Aberer, A. J., Nguyen, L.-T., Minh, B. Q., Von Haeseler, A., & Stamatakis, A. (2015). The phylogenetic likelihood library. *Systematic Biology*, *64*, 356–362.

Foll, M., Fischer, M. C., Heckel, G., & Excoffier, L. (2010). Estimating population structure from AFLP amplification intensity. *Molecular Ecology*, *19*, 4638–4647.

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A bayesian perspective. *Genetics*, *180*, 977–993.

Garrett, G. P., Edwards, R. J., & Hubbs, C. (2004). Discovery of a new population of Devils River minnow (*Dionda diaboli*), with implications for conservation of the species. *The Southwestern Naturalist*, *49*, 435–441.

Gilbert, C. (1978). Type catalogue of the North American cyprinid fish genus *Notropis*. In *Bulletin of the Florida State Museum Biological Sciences* (pp. 1–104). University of Florida.

Gilbert, C. (1998). *Type catalog of recent and fossil North American freshwater fishes: Families Cyprinidae, Catostomidae, Ictaluridae, Centrarchidae and Elassomatidae* (Vol. 1, pp. 1–284). Florida Museum of Natural History, Special Publication.

Girard, C. (1856). Researches upon the cyprinoid fishes inhabiting the fresh waters of the United States, west of the Mississippi Valley, from specimens in the museum of the Smithsonian Institution. In *Proceedings of the Academy of Natural Sciences of Philadelphia* (pp. 165–213). Academy of Natural Sciences of Philadelphia.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution*, *59*, 1633–1638.

Heid, C. A., Stevens, J., Livak, K. J., & Williams, P. M. (1996). Real time quantitative PCR. *Genome Research*, *6*, 986–994.

Hoagstrom, C. W. (2003). Historical and recent fish fauna of the lower Pecos River. In G. P. Garrett & N. L. Allan (Eds.), *Aquatic Fauna of the northern Chihuahuan Desert* (pp. 91–110). Special Publications of Museum of Texas Tech University.

Hubbs, C., Edwards, R. J., & Garrett, G. P. (2008). *An annotated checklist of the freshwater fishes of Texas, with keys to identification of species* (Vol. 60, 2nd ed., pp. 2–87). Texas Academy of Science.

Jelks, H. L., Walsh, S. J., Burkhead, N. M., Contreras-Balderas, S., Diaz-Pardo, E., Hendrickson, D. A., Lyons, J., Mandrak, N. E., McCormick, F., Nelson, J. S., Platania, S. P., Porter, B. A., Renaud, C. B., Schmitter-Soto, J. J., Taylor, E. B., & Warren, M. L., Jr. (2008). Conservation status of imperiled North American freshwater and diadromous fishes. *Fisheries*, *33*, 372–407.

Jombart, T. (2008). *Adegenet*: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*, 1647–1649.

Kollaus, K. A., & Bonner, T. H. (2012). Habitat associations of a semi-arid fish community in a karst spring-fed stream. *Journal of Arid Environments*, *76*, 72–79.

Mayden, R. L., Tang, K. L., Conway, K. W., Freyhof, J., Chamberlain, S., Haskins, M., Schneider, L., Sudkamp, M., Wood, R. M., Agnew, M., Bufalino, A., Sulaiman, Z., Miya, M., Saitoh, K., & He, S. (2007). Phylogenetic relationships of *danio* within the order Cypriniformes: A framework for comparative and evolutionary studies of a model species. *Journal of Experimental Zoology Part B*, *308B*, 642–654.

Meirmans, P. G., & Hedrick, P. W. (2011). Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources*, *11*, 5–18.

Miller, R. R., Minckley, W. L., & Norris, S. M. (2005). *Freshwater fishes of Mexico*. The University of Chicago Press.

NatureServe. (2024). *Notropis amabilis*. NatureServe. https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1194148/Notropis_amabilis Accessed 26 May 2024.

Niemiller, M. L., Graening, G. O., Fenolio, D. B., Godwin, J. C., Cooley, J. R., Pearson, W. D., Fitzpatrick, B. M., & Near, T. J. (2013). Doomed before they are described? The need for conservation assessments of cryptic species complexes using an amblyopsid cavefish (Amblyopsidae: *Typhlichthys*) as a case study. *Biodiversity and Conservation*, *22*, 1799–1820.

Okonechnikov, K., Golosova, O., Fursov, M., & the UGENE team. (2012). Unipro UGENE: A unified bioinformatics toolkit. *Bioinformatics*, *28*, 1166–1167.

O'Leary, S. J., Puritz, J. B., Willis, S. C., Hollenbeck, C. M., & Portnoy, D. S. (2018). These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists. *Molecular Ecology*, *27*, 3193–3206.

Osborne, M. J., Portnoy, D. S., Fields, A. T., Bean, M. G., Hoagstrom, C. W., & Conway, K. W. (2021). Under the radar: Genetic assessment of Rio Grande shiner (*Notropis jemezanus*) and speckled chub (*Macrhybopsis aestivalis*), two Rio Grande basin endemic cyprinids that have experienced recent range contractions. *Conservation Genetics*, *22*, 187–204.

Page, L. E., & Burr, B. M. (2011). *Peterson field guide to freshwater fishes of North America north of Mexico* (2nd ed.). Houghton Mifflin Harcourt.

Perkin, J. S., Starks, T. A., Pennock, C. A., Gido, K. B., Hopper, G. W., & Hedden, S. C. (2019). Extreme drought causes fish recruitment failure in a fragmented Great Plains riverscape. *Ecohydrology*, *12*, e2120.

Perkin, J. S., Troia, M., & Acre, M. (2022). Conservation status of native fishes in the Chihuahuan desert region of the United States: A spatial perspective. In *Proceedings of the Desert Fishes Council Special Publications* (Vol. 2021, pp. 77–103). Desert Fishes Council.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*, e37135.

Pickett, B. D., Wallace, E. M., Ridge, P. G., & Kauwe, J. S. K. (2020). Lingering taxonomic challenges hinder conservation and management of global bonefishes. *Fisheries*, *45*, 347–358.

Platania, S. P., & Altenbach, C. S. (1998). Reproductive strategies and egg types of seven Rio Grande basin cyprinids. *Copeia*, *1998*, 559–569.

Puritz, J. B., Hollenbeck, C. M., & Gold, J. R. (2014). dDocent: A RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ*, *2*, e431.

Quattrini, A. M., Wu, T., Soong, K., Jeng, M.-S., Benayahu, Y., & McFadden, C. S. (2019). A next generation approach to species delimitation reveals the role of hybridization in a cryptic species complex of corals. *BMC Evolutionary Biology*, *19*, 116.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Systematic Biology*, *67*, 901–904.

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Ravaoarimanana, I. B., Tiedemann, R., Montagnon, D., & Rumpler, Y. (2004). Molecular and cytogenetic evidence for cryptic speciation within a rare endemic Malagasy lemur, the northern sportive lemur (*Lepilemur septentrionalis*). *Molecular Phylogenetics and Evolution*, *31*, 440–448.

Seutin, G., White, B. N., & Boag, P. T. (1991). Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, *69*, 82–90.

Sievers, F., Barton, G., & Higgins, D. (2020). Multiple sequence alignment. *Bioinformatics* (4th ed., pp. 227–250).

Stout, C., Schonhuth, S., Mayden, R., Garrison, N. L., & Armbruster, J. W. (2022). Phylogenomics and classification of *Notropis* and related shiners (Cypriniformes: *Leuciscidae*) and the utility of exon capture on lower taxonomic groups. *PeerJ*, *10*, e14072.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*, 3022–3027.

Texas Parks and Wildlife Department. (2012). Texas Conservation Action Plan 2012-2016: Overview. https://www.edwardsaquifer.org/doc_publications/texas-conservation-action-plan-2012-2016-overview/ Accessed 19 June 2024.

Texas Parks and Wildlife Department. (2023). State Wildlife Action Plan: Texas 2023 Comprehensive Revision. https://tpwd.texas.gov/documents/504/State_Wildlife_Action_Plan_for_Texas_2023_USFWS_Final_Submission.pdf Accessed 19 June 2024.

Whitlock, M. C., & Lotterhos, K. E. (2015). Reliable detection of loci responsible for local adaptation: Inference of a null model through trimming the distribution of F(ST). *American Naturalist*, *186*(Suppl 1), S24–S36.

Willis, S. C., Hollenbeck, C. M., Puritz, J. B., Gold, J. R., & Portnoy, D. S. (2017). Haplotyping RAD loci: an efficient method to filter paralogs and account for physical linkage. *Molecular Ecology Resources*, *17*(5), 955–965. https://doi.org/10.1111/1755-0998.12647

Winemiller, K. O., & Rose, K. A. (1992). Patterns of life-history diversification in North American fishes: Implications for population regulation. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*, 2196–2218.

Worthington, T. A., Echelle, A. A., Perkin, J. S., Mollenhauer, R., Farless, N., Dyer, J. J., Logue, D., & Brewer, S. K. (2018). The emblematic minnows of the North American Great Plains: A synthesis of threats and conservation opportunities. *Fish and Fisheries*, *19*(2), 271–307.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Dye, K. R., Fields, A. T., Bean, M. G., Robertson, S. M., Hollenbeck, C. M., Conway, K. W., & Portnoy, D. S. (2025). Assessment of genomic diversity within and between two cryptic shiners, the West Texas shiner (*Notropis megalops*) and the Texas shiner (*Notropis amabilis*). *Journal of Fish Biology*, *106*(3), 836–845. https://doi.org/10.1111/jfb.15999

10958649, 2025, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfb.15999 by Texas A&M University Corpus Christi, Wiley Online Library on [30/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License